ROLE OF MUCIN HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY IN GASTRIC ADENOCARCINOMA

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CERTIFICATE

This is to certify that this dissertation entitled **" ROLE OF MUCIN HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY IN GASTRIC ADENOCARCINOMA"** is the bonafide record work done by **Dr. R. UMA** submitted as partial fulfillment for the requirements of M.D. Degree Examinations Branch III Pathology to be held in APRIL, 2012.

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ABSTRACT

INTRODUCTION

Gastric cancer is the second most common type of cancer world wide. Of the gastric cancer, adenocarcinoma is the most common malignancy. It comprises over 90% of all gastric cancers. Gastric mucins are cytoprotective proteins synthesized by gastric epithelial cells. In general mucins are of two types, neutral and acid mucin. Mucin genes expression in normal stomach includes MUC1 , MUC 5AC in surface epithelium, MUC 6 in deep gastric glands. MUC 2 is not expressed in normal stomach. MUC 2 is expressed in intestinal metaplasia by goblet cells, intestinal type of gastric adenocarcinoma and mucinous gastric adenocarcinoma. MUC 2 expression is decreased in poorly differentiated gastric adenocarcinoma and variable in signet ring cell carcinoma of stomach

AIM : To study the role of mucin histochemistry and immunohistochemistry in gastric adenocarcinoma

MATERIALS AND METHODS

From the period 2008 oct – 2011 sep, 50 cases of gastrectomy specimens were analysed Age, sex and site of the lesion were recorded. Subtyping of carcinoma was done. Mucin type neutral / acidic is identified by AB pH 2.5 PAS and PAS staining. Immunohistochemistry using MUC2 primary antibody was done to assess the role of its expression in various types of gastric adenocarcinoma. Results were tabulated and analysed.

RESULTS

Incidence of gastric cancer among the malignancies during the period 2008 oct – 2011 sep is 4.4% in male – 58% and in female - 42%. Male predominate in the ratio of 3:2 with male peak incidence in the 6^{th} decade and female peak incidence in the 5^{th} decade. Mean age of gastric cancer – 56.7yrs(25-80).

Incidence of early gastric cancer is 2% with commonest site - antropyloric region 86%.Intestinal type predominates by 61.2%. Incidence of signet ring cell carcinoma – 2%. On mucin histochemistry, acid mucin is demonstrated in 96 % of gastric cancer. Acid mucin is expressed more in poorly differentiated and mucinous adenocarcinoma type of gastric cancer. On immunohistochemistry, MUC 2 expression is more in intestinal metaplasia, >50% in mucinous adenocarcinoma, >10% in signet ring cell carcinoma, absent in intestinal type of gastric adenocarcinoma and poorly differentiated adenocarcinoma. AE1/AE3 showed diffuse and strong cytoplasmic positivity in squamous cell carcinoma.

KEYWORDS

Gastric adenocarcinoma, special stain, MUC 2 expression

INTRODUCTION

Gastric cancer is the second most common type of cancer world wide. It is one of the leading cause of death in the world. The highest incidence of gastric cancer is in Asia[59],Central Europe and south America >40/1,00,000. The lower rates are in North America,Northern Europe, most countries in Africa and south eastern Asia <15/1,00,000[58].

In India, it is around 8.9/1,00,000. Thus the incidence of gastric carcinoma varies from place to place due to environmental factors, dietary and host related factors[81].

Of the gastric cancer, adenocarcinoma is the most common malignancy. It comprises over 90% of all gastric cancers. Gastric carcinoma is more common in low socioeconomic groups and in individuals with multifocal mucosal atrophy and intestinal metaplasia.

The overall incidence of gastric adenocarcinoma is decreased world wide but the cancer of cardia is on the rise[56].

Gastric mucins are cytoprotective proteins synthesized by gastric epithelial cells. In general mucins are of two types, neutral and acid mucin. Normal gastric mucin is neutral mucin. There is transition from neutral mucin to acid mucin when there is neoplastic transformation.

Mucin genes expression in normal stomach includes MUC1 ,MUC 5AC in surface epithelium, MUC 6 in deep gastric glands. MUC 2 is not expressed in normal stomach. MUC 2 is expressed in intestinal metaplasia by goblet cells, intestinal type of gastric adenocarcinoma and mucinous gastric adenocarcinoma[73].

MUC 2 expression is decreased in poorly differentiated gastric adenocarcinoma and signet ring cell carcinoma of stomach. MUC 2 expressing goblet cells are stained by Alcian blue pH 2.5. [67]

This prospective study of gastrectomy cases was done with special reference to mucin expression in various types of gastric adenocarcinoma. The patient details were collected and histopathological evaluation of gastrectomy specimens were done with routine H & E stain and special stains to demonstrate the nature of mucin expressed in it.

In semiurban area like Thanjavur, the life style and nutrition factor proves to be vital in the occurrence of gastric carcinoma. In this study , the histopathological features of gastric adenocarcinoma was described in detail paying particular attention to the expression of mucin. The mucin profile in gastric adenocarcinoma was studied by mucin histochemistry with PAS , Alcian blue pH 2.5 PAS staining and with immunohistochemistry by MUC 2 (Leica , USA) expression. The cases include mucinous adenocarcinoma , signet ring cell carcinoma and well differentiated, moderately differentiated, poorly differentiated adenocarcinoma along with areas of intestinal metaplasia.

Recent studies and literature proved that MUC gene expression in gastric adenocarcinoma and its precursors serve as diagnostic and prognostic marker.

A case of squamous cell carcinoma in the cardiac region of stomach was studied with AE1/AE3 expression by immunohistochemistry.

This study is undertaken in view of evaluating the actual incidence of gastric carcinoma in semiurban area like Thanjavur with particular attention to mucin expression. In addition the recent literatures, journals and research publications regarding gastric cancer were also immensely reviewed.

AIMS AND OBJECTIVES

Gastrectomy and endoscopic biopsies of stomach were studied to find out

1. Incidence of gastric adenocarcinoma in relation to age and sex
2. Site of occurrence (cardia , body , antrum)
3. Role of mucin histochemistry in various types of gastric adenocarcinoma by Alcian blue pH 2.5 PAS and PAS.
4. Expression of MUC 2 , a mucin protein studied by immunohistochemistry on normal stomach mucosa , intestinal metaplasia and various types of gastric adenocarcinoma.
5. To analyse mucin association with respect to subtypes based on degree of differentiation of gastric adenocarcinoma
6. To analyse the prognosis of various types of gastric adenocarcinoma by MUC 2 expression.

MATERIALS AND METHODS

A total of 303 endoscopic biopsies of stomach and 50 gastrectomy specimens including total and partial gastrectomy were received for examination in the Department of Pathology, Thanjavur medical college from medical and surgical gastroenterology department from 2008 October to 2011 September were included in this study.

For all the cases, details of age and sex were recorded. Depending on the site of growth, stomach was opened through the greater or lesser curvature. The specimen is pinned out on a wax board with mucosal side up and fixed in 10% buffered formalin overnight. The specimen is measured including the length of greater and lesser curvature. The location, shape, maximal dimension of the tumor and its distance to margins are recorded. Any other gross abnormalities of gastric mucosa also be recorded. The grossly identified tumor is then cross sectioned to examine the depth of invasion.

SECTIONS FOR HISTOLOGY[1] INCLUDE

1. Tumor - four sections through wall, including tumor border and adjacent mucosa
2. Non neoplastic mucosa, mid stomach , two sections
3. Proximal line of resection along lesser curvature, two sections
4. Proximal line of resection along greater curvature , two sections
5. Distal line of resection (along pylorus and duodenum, if present), two sections
6. Spleen , if present
7. Pancreas , if present
8. Lymph nodes:

a. Pyloric, Lesser curvature , Greater curvature

b. Omentum, Perisplenic

Bits were processed routinely for paraffin embedding. Multiple thin sections of 3-5µ thickness were cut from paraffin blocks and stained with routine H& E stain.(Appendix I)

Blocks that had areas of intestinal metaplasia and frank malignancy were taken and stained with special stains such as PAS (Appendix III) and Alcian Blue pH 2.5 PAS (Appendix II). A subjective assessment of relative proportion of acidic and neutral mucin was made for each tumor by Alcian Blue pH 2.5 PAS. Samples of appendix and colonic mucosa were taken as control for PAS and AB pH 2 .5 PAS respectively.

Blocks of signet ring cell carcinoma, mucinous adenocarcinoma and well differentiated adenocarcinoma, moderately differentiated, poorly differentiated adenocarcinoma along with areas of intestinal metaplasia were taken and studied for MUC 2 expression by immunohistochemistry (Appendix IV) . Expression of AE1/AE3 in squamous cell carcinoma of stomach was also studied.

REVIEW OF LITERATURE

Gastric cancer is a leading cause of death in the world inspite of a trend of decreasing incidence in most countries. Gastric adenocarcinoma has high mortality rate with 5yrs survival rate of approximately 20%.[65] One of the main survival limiting factor is late detection of tumor.

ANATOMY[80]

Stomach is a distensible bag with a variable size located a few centimeter below the diaphragm. By convention it is divided into 5 regions. The cardia is an illdefined area that connects the gastroesophageal junction. The fundus is the superior portion of the stomach above GE junction. The body or corpus is the main portion of the stomach below the fundus. The antrum is the distal portion separated from the body approximately at the incisura angularis. Finally , the pylorus is a 1-2 cm narrow channel that extends from the antrum and connects the stomach to the duodenum.

Stomach is a complex organ particularly in its epithelial components . Its mucosa is divided into fundic and antral type. Fundic type mucosa is present in fundus and body. It consists of fundic or oxyntic glands occupying approximately 80% of the mucosal thickness. The superficial [20%] consists of foveolar cells that are tall, columnar and produce neutral mucin. The fundic glands contain acid secreting [parietal cells] and zymogenic cells [chief cells].

The antral type mucosa is seen in antrum, pylorus and cardia where the deeper glands are loosely packed and mucin producing. In antral type mucosa, the ratio of mucinous glands to overlying foveolae is roughly 1:1. The lamina propria of the stomach contains only a minimal number of lymphocytes, plasma cells, eosinophils and mast cells.

The submucosa consists of loose connective tissue with numerous elastic fibres. It contains arteries, veins ,lymph vessels and Meissner's nerve plexus. The muscuaris propria and serosa of the stomach are histologically similar to those of stomach. The muscularis propria is formed of inner circular and outer longitudinal layer.

EPIDEMIOLOGY[80]

Gastric cancer incidence varies with geography. In Japan, Chile, Coast Rica and Eastern Europe the incidence is upto 20 fold higher than in North America, Northern Europe, Africa and South east Asia. Due to mass endoscopic screening program in the high incidence region such as Japan, 35% of newly detected cases were early gastric cancer ie; tumor limited to mucosa and submucosa.

In United states ,the incidence was reduced to 85% in the 20th century. Gastric adenocarcinoma was the commonest cause of cancer death during 1930s and remains a leading cause of cancer death world wide. Now it accounts for fewer than 2.5% of cancer deaths in the United states. Similar declines have been reported in many other Western countries, suggesting that environmental and dietary factors are responsible.

Even though the overall incidence of gastric cancer is reduced, cancer of gastric cardia is on the rise. It is due to Barrett's esophagus, chronic gastric esophageal reflux disease and obesity due to common pathogenesis, esophageal adenocarcinoma and gastric cardia adenocarcinoma are similar in morphology, clinical behavior and therapeutic response.

AGE AND SEX DISTRIBUTION[54]

Gastric carcinoma is extremely rare below the age of 40. It increases thereafter to reach highest rate in the oldest age group both in male and female. The intestinal type rises faster than the diffuse type which is more common in males than in females.

Diffuse type affects younger individuals mainly females and has poor prognosis.

AETIOLOGY[54]

HIGH RISK – low socioeconomic status, salt intake, smoked meat or fish, pickled vegetables, chilli , peppers, soyabeans, host factor – H.pylori infection

HIGH RISK EXPLANATION

The diets mentioned above have low level of micronutrients , vitamins and antioxidants which favors intraluminal formation of genotoxic agents such as specific N – nitrosocompounds that leads to gastric carcinoma

H.pylori

Long standing infection by H.pylori leads to chronic gastritis, atrophic gastritis and intestinal metaplasia which is associated with increased risk of intestinal type of gastric adenocarcinoma.

Incidence of gastric adenocarcinoma of diffuse type is higher in blood group A, in people having family history of gastric carcinoma or pernicious anemia.

LOWEST RISK

Fresh fruits, vegetables, ascorbic acid, carotenoids, folates and tocopherols

YOUNG AGE

In contrast to intestinal type, diffuse type is more common in young age with equal incidence in both high risk and low risk geographic areas due to regulation by genetic factors

PATHOGENESIS[65]

While majority of gastric cancers are not hereditary, the mutation identified in familial gastric cancer has provided important insights into the mechanism of carcinogenesis in sporadic cases, germline mutations in CDH1, which encodes E – cadherin, a protein that contributes to epithelial intercellular adhesion. It is usually associated with familial gastric cancer which is usually of diffuse type. Mutation in CDH1 are usually present in about 50% of sporadic cases of diffuse gastric cancer. E- cadherin expression is decreased in the rest often by methylation of the CDH1 promoter. Thus the loss of E – cadherin function seems to be a key step in the development of diffuse gastric cancer. Individuals with BRCA 2 mutations are at increased risk of developing diffuse gastric cancer.

In intestinal type of gastric cancer, there is mutation of β catenin, a protein that binds to both E cadherin and APC. There is also microsatellite instability and hypermethylation of several genes including TGFβRII, BAX, IGFRII and INK 4a/p16 in sporadic intestinal type of gastric cancer.

Genetic variants of proinflammatory and immune response , including those that encode IL - 1β, TNF, IL – 10, IL -8 and TLR 4 [Toll like receptors 4] are associated with increased risk of gastric cancer when accompanied by H.pylori infection and p53 mutation is present in majority of sporadic gatric cancer of both histologic types. Thus chronic inflammation promotes gastric cancer.

LOCALISATION

Most common site is distal stomach in antropyloric region and along the lesser curvature , recently the incidence Is more common in cardiac region of stomach. Carcinoma in the corpus is located along the greater curvature or lesser curvature. Early cancer occur commonly in middle part of stomach along the lesser curvature. Advanced cancer occur more commonly in antral region followed by corpus region.

CLINICAL FEATURES

Early cancer is usually asymptomatic, 50% present with dyspepsia. In advanced cancer patient present with abdominal pain which is not relieved by food, if ulcerated there will be hemetemesis .If the tumor obstruct the gastric outlet, there will be vomiting. Systemic symptoms such as anorexia, weight loss suggest disseminated disease

PRECURSORS[54]

The precursors of gastric cancer have been separated into 2 major categories

1. Precancerous conditions – clinical condition with increased risk of gastric cancer
2. Precancerous lesions - pathological changes from which gastric carcinoma eventually evolves .

It is believed that precancerous condition is preceded by the occurance of precancerous lesion.

PRECANCEROUS CONDITIONS

Epithelial polyp

Chronic atrophic gastritis - more common condition leads to carcinoma

Intestinal metaplasia

Chronic ulcer

Gastric remnants

Hyperplastic gastropathy

INTESTINAL METAPLASIA

The gastric mucosa is transformed into intestinal type mucosa with complex and heterogenous features.

Intestinal metaplasia begins in the neck region which is the proliferative zone of normal gastric glands and first appears at antral corpus junction.

Charles M leys et al studied that two types of metaplasia is associated with gastric cancer, namely intestinal metaplasia and antralization of gastric fundus.

CLASSIFICATION OF INTESTINAL METAPLASIA

Based on cell type and their functional features

1. complete intestinal metaplasia

Gastric mucosa assumes appearance of small intestine without villi. Glands are lined by absorptive cells, goblet cells, paneth cells and endocrine cells. Mucin can be sulfomucin, sialomucin or both.

2. Incomplete intestinal metaplasia

Instead of absorptive cells, columnar cells between the goblet cells resemble foveolar mucous cells. Mucin can be neutral, sialomucin or sulfomucin.

JASS AND FILIPE CLASSIFICATION

Based on presence of absorptive cells in complete type and mucus secreting columnar cells in incomplete type

TYPE I – complete intestinal metaplasia

TYPE II – incomplete type , Type II A – nonsulphated mucin

Type II B - Sulfated mucin

TYPE II - more prone for precancerous situations and gastric adenocarcinoma.

RECENT CLASSIFICATION[54]

TYPE I - complete intestinal metaplasia

TYPE II - incomplete intestinal metaplasia [old type II A]

TYPE III - incomplete intestinal metapasia with predominant sulfated mucin [old type IIB]

PRECANCEROUS LESIONS

Adenoma with dysplastic cells is the most common condition. Dysplasia is closely associated with expanding or intestinal type of gastric cancer

INTRA EPITHELIAL NEOPLASIA

Intraepithelial neoplasia or dysplasia arises in either the native gastric or of intestinalized gastric epithelia. Pyloric gland adenoma is a form of intraepithelial neoplasia arising in the native mucosa. In the multistage theory of gastric oncogenesis, intraepithelial noeplasia lies between atrophic metaplastic lesions and invasive cancer. It has to be differentiated from reactive/regenerative changes associated with inflammation and invasive carcinoma. several proposals have been made for terminology of the morpholo gical spectrum of lesions that lies between non neoplastic changes and early invasive cancer, including international Padova classification.

INDEFINITE FOR INTRAEPITHELIAL NEOPLASIA

Cases lacking all the features for definitive diagnosis of intraepithelial neoplasia may be placed in this category. In native gastric mucosa, foveolar hyperplasia may be indefinite for dysplasia ,showing irregular and tortuous tubular structures with epithelial mucus depletion, high nuclear- cytoplasmic ratio and loss of cellular polarity. Large , oval/round, hyperchromatic nuclei associated with prominent mitosis are usually located near proliferative zone in the mucus neck region. In intestinal metaplasia, areas indefinite for intraepithelial neoplasia exhibit a hyperproliferative metaplastic epithelium. The glands may appear closely packed, lined by cells with large , hyperchromatic rounded or elongated, basally located nuclei. Nucleoli are an inconstitent finding. The cytoarchitectural alteration tend to decrease from the base of the glands to their superficial portion.

INTRAEPITHELIAL NEOPLASIA

It has flat, polypoid or slightly depressed growth pattern. In Western countries, the term adenoma is applied for discrete, protruding lesion. In Japan, adenoma include all gross types such as flat, elevated and depressed. Gasric adenoma are less common than hyperplastic polyp and accounts for about 10% of polyps. They arise in the antrum/mid stomach and in areas of intestinal metaplasia.

LOW GRADE INTRAEPITHELIAL NEOPLASIA

It shows tubular structures with budding and branching, papillary infolding, crypt lengthening with serration and cystic changes . Glands are lined by enlarged columnar cells, with minimal or no mucin. Homogenously blue vesicular, rounded/ ovoid nuclei are usually pseudostratified in the proliferation zone located at the superficial portion of the dysplastic epithelium.

HIGH GRADE INTRAEPITHELIAL NEOPLASIA

There is increasing architectural distortion with glandular crowding and prominent cellular atypia. Tubules can be irregular in shape with frequent branching and folding. There is no stromal invasion. Mucin secretion is minimal or absent. The pleomorphic, hyperchromatic, usually pseudostratified nuclei often are cigar shaped. Prominent amphophilic nucleoli are common. Increased proliferative activity is present through out the epithelium.

PROGRESSION OF INTRAEPITHELIAL NEOPLASIA TO CARCINOMA[81]

Carcinoma is diagnosed when the tumor invades into the lamina propria (intramucosal carcinoma) or through the muscularis mucosa. Upto 80% of intraepithelial neoplasia may progress to invasion

CORREA CASCADE of multistep carcinogenesis[56]

The development of gastric adenocarcinoma represents the involvement of

Inflammation

⬇

Intestinal metaplasia

⬇

Dysplasia

⬇

Gastric cancer

CLASSIFICATION OF GASTRIC ADENOCARCINOMA[54]

Gastric carcinoma is classified according to their site , gross and histomorphology.

Based on invasiveness - 2 types

I. Early gastric cancer

Invasive adenocarcinoma of stomach confined to the mucosa or submucosa regardless of lymphnode metastasis.

This type has male predominance, occurs in >50 yrs of age, usually asymptomatic or present with epigastric pain, dyspepsia. Present as small mass measuring around 2 – 5 cm on lesser curvature of angularis region.

Divided into 3 types based on endoscopic appearance

1. Type I – protruding
2. Type II – superficial
   - a - elevated
   - b - flat
   - c - depressed
3. Type III - excavating

Majority of early gastric cancer are well differentiated tubular or papillary variants

II. late gastric carcinoma

Invasion of tumor into muscular wall

TNM CLASSIFICATION OF GASTRIC TUMORS[81]

T – primary tumor

TX – primary tumor cannot be assessed

T0 – no evidence of primary tumor

Tis - Carcinoma in situ: intraepithelial tumor without invasion of lamina propria

T1 – Tumor invades lamina propria or submucosa

T2 – Tumor invades muscularis propria or subserosa

T3 –Tumor penetrates serosa [visceral peritoneum] without invasion of adjacent organ

T4 –Tumor invades adjacent structures such as spleen, transverse colon , liver, diaphragm, Pancreas, adrenal, abdominal wall, kidney, small intestine and retroperitoneum.

N – Regional lymph node

NX - regional lymph node cannot be assessed

NO – no regional lymph node metastasis

N1 – Metastasis in 1- 6 regional lymph nodes

N2 – Metastasis in 7 – 15 regional lymph nodes

N3 –Metastasis in more than 15 regional lymph nodes.

M – Distant metastasis

MX – Distant metastasis cannot be assessed

M0 – NO distant metastasis

M1 – Distant metastasis

STAGE GROUPING[81]

Stage 0 -	Tis	N0	M0
Stage I A –	T1	N0	M0
Stage IB -	T1	N1	M0
	T2	N0	M0
Stage II -	T1	N2	M0
	T2	N1	M0
	T3	N0	M0
Stage IIIA -	T2	N2	M0
	T3	N1	M0
	T4	N0	M0
Stage IIIB -	T3	N2	M0
Stage IV -	T4	N1,N2,N3	M0
	T1,T2,T3	N3	M0
	Any T	Any N	Any M1

BORRMANN CLASSIFICATION[54]

Based on macroscopic appearance it is of 4 types

Type I - polypoid cancer, occurs on corpus along greater curvature

Type II - fungating type, occurs on antrum along lesser curvature

Type III - ulcerating type, occurs on corpus along greater curvature

Type IV - diffusely infiltrating type or linitus plastica , stomach has leather bottle appearance

Type II and III are more common. Mucinous adenocarcinoma appears gelatinous and glistening on cut surface.

Based on degree of differentiation, it is of 3 types

1. well differentiated

   >95% of tumor composed of glands

2. Moderately differentiated

   50% - 95% of tumor composed of glands

3. Poorly differentiated

   <50% of tumor composed of glands

LAUREN CLASSIFICATION

1. Intestinal
2. Diffuse
3. Mixed

INTESTINAL TYPE

This type has features resembling differentiated colonic carcinoma, characterized by recognizable glands that range from well to moderately differentiated with more

inflammation. This type of tumor arise from the background of intestinal metaplasia, can also be associated with atrophic gastritis , dysplasia in adjacent mucosa.

Mucinous phenotype can be intestinal , gasric or gastrointestinal.

DIFFUSE TYPE

This type is composed of poorly cohesive cells diffusely infiltrating into the gastric wall with little or no gland formation. Individual cell is small, round , arranged in single or in clusters. These cells can also be arranged in abortive, lacy gland like or in reticular pattern. This type resembles as signet ring cell tumor in WHO classification.

It has low mitosis than intestinal tumor. There will be small interstitial mucin, more desmoplasia and less inflammation.

WHO classification

Adenocarcinoma – intestinal, diffuse

Papillary adenocarcinoma

Tubular adenocarcinoma

Mucinous adenocarcinoma

Signet ring cell carcinoma

Adenosquamous carcinoma

Squamous cell carcinoma

Small cell carcinoma

Undifferentiated carcinoma

Others

TUBULAR ADENOCARCINOMA

It is composed of prominent dilated or slit like and branching tubules varying in their diameter, acinar like structures are also present. Individual cells are columnar , cuboidal or flattened by intraluminal mucin. Clear cells may be seen. Cytologic atypia varies from low grade to high grade. The poorly differentiated variant is called solid carcinoma. Tumor with a prominent lymphoid stroma is called medullary carcinoma or carcinoma with lymphoid stroma.

PAPILLARY ADENOCARCINOMA

It is a well differentiated exophytic tumor with elongated finger like process lined by cylindrical or cuboidal cells supported by fibrovascular connective tissue cores. The cells maintain their polarity. Some show tubular differentiation [papillotubular]. There will be severe nuclear atypia. Tumor edge is sharply demarcated from the surrounding areas

MUCINOUS ADENOCARCINOMA

This type is identified by the presence of extracellular mucin which constitutes >50% 0f tumor areas. It has two major growth patterns

1. glands lined by a columnar mucous secreting epithelium with interstitial mucin
2. chains or irregular clusters of malignant cells floating freely in mucinous lakes

scattered signet ring cells are also present.

SIGNET RING CELL CARCINOMA

>50% of tumor consist of isolated or small groups of malignant cells containing intracytoplasmic mucin. Signet ring cells may also form delicate trabecular, glandular or solid pattern. Signet ring cell carcinoma are infiltrative with more desmoplasia. special stains used to express the mucin are PAS, Alcian blue and mucicarmine

Tumor cells have 5 morphological features[81]

1. Signet ring cells - cells with nuclei pushed against cell membrane creating classical signet ring

appearance due to an expanded , globoid , optically clear cytoplasm. These cells contain acid mucin which is stained by alcian blue at pH 2.5

2. Histiocytoid - other diffuse cancer contain cells with central nuclei resembling histiocytes with little or no mucin
3. Eosinophilic - small deeply eosinophilic cells with prominent but minute cytoplasmic granules containing neutral mucin
4. Small mucin poor cells - small cells with little or no mucin
5. Anaplastic cells with little or no mucin

NEUROENDOCRINE DIFFERENTIATION IN ADENOCARCINOMA[1]

Can be placed in one of the following category:

1. well differentiated and slow growing well differentiated neuroendocrine tumors composed of neuroendocrine cells of the gastric mucosa.
2. Tumors with morphological features of neuroendocrine differentiation such as trabeculae, rosettes, insular, dense core secretory granules ultrastructurally; immunoreactive for NSE (neuron specific enolase) and other neuroendocrine markers.

Tumors with features of large cell neuroendocrine carcinoma of lungs have worst prognosis

3. Small cell carcinoma are morphologically analogous to pulmonary counterpart with aggressive clinical course.
4. Other wise typical adenocarcionma of either diffuse or intestinal type having cells

that exhibit argyrophilic or some other phenotypical attribute of neuroendocrine cells 2^{nd} and 3^{rd} categories are common.

OTHER RARE VARIANTS

ADENOSQUAMOUS CARCINOMA

It has combined expression of both adenocarcinoma and squamous cell carcinoma. If there is a distinct boundary between the two components, then it is called collision tumor. Tumor with discrete foci of benign appearing squamous metaplasia are termed adenocarcinoma with squamous differentiation [adenoacanthoma]

SQUAMOUS CELL CARCINOMA

Pure squamous cell carcinoma is rare in stomach. It resembles squamous cell carcinoma arising elsewhere in the body.

.**UNDIFFERENTIATED CARCINOMA**

Belongs to intermediate group of Laurens classification and it lacks any differentiated features

OTHER RARE TUMORS IN STOMACH

Mixed adenocarcinoma and carcinoid

Small cell carcinoma

Parietal cell carcinoma

Choriocarcinoma

Endodermal sinus tumor

Embryonal cell carcinoma

Paneth cell rich adenocarcinoma

Hepatoid adenocarcinoma

JAPANESE CLASSIFICATION

Includes 2 categories - common type and special type

COMMON TYPE

Include papillary , tubular , mucinous and signet ring cell carcinoma

SPECIAL TYPE

Adenosquamous carcinoma, squamous cell carcinoma and carcinoid

Poorly differentiated can be solid or non solid type

In addition to tumor typing, this classification includes status of lymphatics, venous penetration, tumor invasion, cancer stroma relation, pattern of tumor growth, hepatic, peritoneal metastasis and clinical / operative features

MING CLASSIFICATION

Based on tumor growth and invasiveness , it is of 2 types

1. **Expanding type**

This type has growth in cohesive nodules, fungating or polypoid mass with sharply defined periphery compressing the neighboring tissue . This type is usually associated with chronic atrophic gastritis, intestinal metaplasia and dysplasia. This tumor is composed of large glands, more lymphocytic infiltration and less desmoplastic response. E cadherin is preserved in this tumor which is a cell adhesion molecule. On electron microscopy , well developed desmosomes are present. This type constitutes 67% of gastric tumor.

2. **Infiltrative type**

This type has indistinct tumor boundry. It shows infiltration by individual cell or as small glands. Cell adhesion molecule E cadherin is lost. On electron microscopy, there is loss of desmosomes. There is more desmoplasia than that of expanding type

This classification is adapted for clinical usage and image analysis. Expanding type has better prognosis than infiltrative type. Ming and Lauren classification have similarities. Intestinal type are similar to expanding type Diffuse type are similar to infiltrative type.

NAKAMURA'S CLASSIFICATION

1. Differentiated
2. Undifferentiated

   Includes poorly differentiated adenocarcinoma, signet ring cell carcinoma, Mucinous carcinoma

MULLIGAN CLASSIFICATION

On the basis of cell type :

1. Mucus cell type (46.7%)
2. Pylorocardiac gland cell type (29.7%)
3. Intestinal cell type (23.6%)

GOSEKI'S CLASSIFICATION

It is of four types based on tubular differentiation and amount of intracytoplasmic mucin

Group I - well tubular differentiation but poor mucin

This group constitutes around 40% of gastric adenocarcinoma

Group II – well tubular differentiation but rich mucin

This group constitutes around 3.5% of gastric adenocarcinoma

Group III – poor tubular differentiation with poor mucin

This group constitutes around 20% of gastric adenocarcinoma

Group IV – poor tubular differentiation but rich mucin

This group constitutes around 36.5% of gastric adenocarcinoma

Group I is more prone for liver metastasis

Group III is usually an intermediate finding

Group IV is more prone for lymph node metastasis, peritoneal dissemination and direct invasion of adjacent organ.

CARNEIRIO CLASSIFICATION

1. Glandular Pattern
2. solid pattern - better prognosis [according to WHO it has poor prognosis]
3. isolated
4. mixed cell type

it is around 30% of gastric adenocarcinoma and has worse prognosis

ADACHI CLASSIFICATION

On the basis of prognosis

BETTER PROGNOSIS

Tubular adenocarcinoma

Solid / medullary adenocarcinoma

Well differentiated adenocarcinoma

Mucinous adenocarcinoma

POOR PROGNOSIS

Signet ring cell carcinoma

Poorly differentiated schirrous carcinoma

Poorly differentiated mucinous carcinoma

JASS CLASSIFICATION

Gastric type

Intestinal type

FROM VARIOUS STUDIES

EXTREMELY WELL DIFFERENTIATED ADENOCARCINOMA STOMACH [EWDA]

Takashi yao et al[75] showed that Extremely well differentiated adenocarcinoma [EWDA] is a neoplastic condition composed of highly differentiated neoplastic epithelium which mimics normal gastric mucosa or intestinal metaplastic mucosa with mild nuclear atypia but has the ability to invade the gastric wall.

EWDA constitutes 1% of gastric cancer, mean age [45-81yrs] 62 yrs, it mimics like neoplastic or dysplastic lesions in the stomach. It is usually located in the upper or middle third of the stomach and it also has both gastric and intestinal phenotype . Histologically too bland and too similar to benign foveolar epithelium. This tumor is similar to that of adenoma malignum of uterine cervix.

MICROPAPILLARY CARCINOMA

Dae woon eom et al[14], studied a rare variant of gastric cancer called micropapillary carcinoma[MPC] identified by small clusters of tumor cells in the clear lacunar space mimicking lymphatic or vascular channels.MPC constitutes 6.4 % of gastric cancer.

SPREAD OF GASTRIC CANCER[81]

Distal carcinoma of stomach invade duodenum in high percentage of cases. Carcinoma of proximal stomach involves the esophagus. The serosal spread of the tumor is more common in infiltrative type of gastric cancer than expanding type.

Local extension also occurs in the omentum, colon, pancreas and spleen. The mucosal and submucosal - Borrman's lymphatic plexus of the stomach is often invaded . From here, the tumor spread to perigastric, periaortic and celiac axis related lymph nodes. Distal third involves the hepatoduodenal nodes. The mucosal lymphangiectasia associated with regional lymph node metastasis. Invasion of tumor into blood vessel wall is called vasculitis carcinomatosa.

The most frequent site of distant metastasis are liver, peritoneum, lungs, adrenal glands and ovary. Bilateral metastasis of the tumor to the ovary is called Krukenberg's tumor. Metastasis also occurs in uterine body and cervix.

PROGNOSIS[1]

1. Gastric carcinoma in the young age is predominantly of diffuse type and it has poor prognosis.
2. Tumor stage - the depth of invasion into the serosa has more tendency to spread to lymph node. This type has poor prognosis.

Shigang ding M et al[69], lymphatic invasion is the source of lymph node metastasis in gastric cancer extending over submucosal layer. It has to be differentiated by retraction artifact that isolate tumor aggregates due to tissue shrinkage during fixation.

3. Tumor in cardia,fundus or esophago gastric junction has poor prognosis
4. Tumor with expanding or pushing margin have better prognosis than that of diffuse infiltration type.
5. Small tumor size is associated with better prognosis since they are associated with depth of invasion.
6. On the basis of various types, the decreasing order of prognosis is that of

High grade carcinoma – adenosquamous, anaplastic and neeuroendocrine carcinoma; diffuse and mixed; glandular pattern

7. The infiltration of inflammatory cells between the tumor tissue has good prognosis.

8. Tumor with perineural invasion has poor prognosis

9. If tumor is found at the limit of excision, there is more chance of reccurances of the tumor.

10. Negative lymph node status has 5 years survival in 50% of cases . with nodal involvement the survival rate decreases to 10% .

11. Radical subtotal gastrectomy and radical lymph adenectomy has better survival than other types of surgery.

12. c-erB 2 protein expression is an independent indicator of poor prognosis.

13. p53 expression is associated with decreased survival.

14. Increased expression cathepsin D is associated with decreased survival. cathepsin B and cathepsin L expression is associated with tumor invasion and metastasis.

15. p27Kip 1 expression is associated with decreased survival.

16. Loss of Fhit protein is associated with decreased survival.

17.Expression of T antigen in MN blood system is said to correlate with depth of invasion and metastatic spread.

Shigang ding M et al[69] studied that microvessel density is a prognostic indicator in a variety of human malignancies with increased micro vessel density correlating with shorter overall and relapse free survival rate. It is identified by CD 105 + associated with blood vessel invasion and distant metastasis of tumor. Microvessel is regular and well formed in gastritis, dilated and irregular in hyperplastic polyp. In gastric cancer,

microvessel is irregular , dilated and immature.

MUCIN PROFILE IN STOMACH

Gastric mucins are critical cytoprotective proteins synthesized by gastric epithelial cells. Mucins are high molecular weight glycoprotein that are synthesized by secretory epithelial cells as membrane bound or secreted products[20].

Mucins are characterized by a tandem repeat region rich in threonine / serine which are o-glycosylation sites. Each mucin is distinct due to difference in tandem repeat sequence length and has unique non repetitive sequence[67].

In general mucins are classified into neutral and acid mucin, of which acid mucins are of 2 types - 1. sulphated / sulphomucin 2.carboxylated / sialomucin[67]

Normal gastric mucin is of neutral type. Small amount of acid mucins such as sialomucin, sulphomucin are produced in foveola, neck cells of the fundus, foveola of antrum and cardiac glands of stomach[73,67].

Neutral mucin production is decreased in neoplastic transformation of gastric mucosa. The transition of neutral to acid mucin occurs in intestinal metaplasia which is a common precursor condition of stomach carcinoma[67]. The mucin that is produced during the transition stage and gastric adenocarcinoma is predominantly of sulphomucin, an acid mucin.

In well differentiated adenocarcinoma, it is predominently of sulphomucin , which is characteristic of mature surface mucin cells. In moderately differentiated adenocarcinoma and poorly differentiated adenocarcinoma , there is predominantly sialomucin which is characteristic of intestinal goblet cells[20].

In mucinous adenocarcinoma , the mucin secreted is acidic mucin – o acylated form of sialomucin. This variant has good prognosis than that of signet ring cell

carcinoma of stomach[18],

Acid mucin and neutral mucin are clearly identified by special stain studies such as PAS – periodic acid Schiff, combined alcian blue pH 2.5 PAS.

MUCIN GENE EXPRESSION IN NORMAL GASTRIC MUCOSA AND GASTRIC ADENOCARCINOMA

Human gastric epithelium has an unique mucin gene pattern which becomes markedly altered in preneoplastic and neoplastic conditions. More than fifteen mucin genes have been identified. They are categorized into

1. *Membrane associated mucin*

   MUC 1, MUC 3, MUC 4, MUC 12, MUC 16, MUC 17

2. *Gel forming mucin*

   MUC 2, MUC 5AC, MUC 5B, MUC 6

   Gel forming mucin gene is on chr 11p15.5

3. *Soluble form*

   MUC 1N - MUC 7

In normal stomach there is increased expression of

MUC 1, MUC 5AC in surface epithelium. MUC 6 in deep gastric glands. **MUC 2 is not normally expressed[20,62,67,73].**

MUC 1[(73)]

Expressed in apex of the cell, It has inhibitory role in cell to cell adhesion, cell to stromal interaction and cytotoxic immunity. MUC 1 functions as signal transducer interacting with EGFR and participates in carcinogenesis. It is a marker for aggressiveness.

MUC 2[73,67]

It is expressed in intestinal type secretory mucin or goblet type or gel forming mucin Normally it is expressed in goblet cells. It act as a protective barrier and has tumor suppressor properties. It is responsible for the indolent behavior of the tumor. Since it is a gel forming mucin it act as a containing factor preventing the spread of cells. It is commonly expressed in intestinal differentiation of gastric adenocarcinoma. It shows diffuse intracytoplasmic positivity.

Mucin 2 gene expression

Takayuki seki et al[76], studied that MUC 2 a glycoprotein known as intestinal mucin related protein antigen ,expressed in goblet cells including metaplasic cells in stomach other parts of alimentary tract.

Subramani duraibabu et al[73], studied that MUC5AC and MUC 6 are expressed in normal stomach mucosa. MUC 2 is not expressed in normal stomach mucosa.

Samuel et al[67] studied that the process of neoplastic transformation in the stomach is associated with decrease in expression of these mucin and there is increased expression of mucin genes such as MUC2, MUC3 ,MUC4 which is normally expressed by intestine.

Advanced stage gastric cancer expresses more mucin genes compared to that of less differentiated and early stage of gastric cancer. He studied that gastric cancer frequently demonstrate 3 types of alterations

1. Loss of normal mucin gene expression
2. Increased mucin core peptide immunoreactivity
3. Expression of mucin core peptide and mRNA which is not found in corresponding normal epithelium

The transition from MUC 5 and MUC6 mucin gene expression in normal gastric mucosa to MUC 2 and MUC3 mucin gene in intestinal metaplasia is associated with appearance of new carbohydrate antigen.

Samuel et al[67] studied that

1. Expression of multiple mucin secondarily reflect increased dedifferentiation and genetic alteration found in advanced gastric cancer.
2. Increased mucin gene expression may contribute to tumor cell growth and metastatic abilities

Takayuki seki et al[76], MUC 2, a sialomucin which is otherwise called intestinal mucin related protein antigen. It is a major colonic apomucin expressed in goblet cells.

Emmanuelle leteurtre et al[18], showed that MUC 2 gene is located on chr11p15.5

Ackerman et al[1], showed that MUC 2 gene corresponds to sialomucin which is an acid Mucin not normally expressed in normal stomach. In this study,

Minh d.nguyen et al[55], studied that MUC 2 secretory mucin gene plays first line defense mechanism by protecting epithelial surface and initiating host immune response.

Dabbs[13] – since it is a gel forming mucin, it act as a containing factor preventing the spread of cells

MUC 5AC[63]

It is otherwise called HGM or human gastric mucin. It is normally expressed in foveolar epithelium and mucus neck cells in antrum, cardiac and fundus. It is located in supra or perinuclear areas.

MUC 6

It is normally expressed in cells of fundus ,glandular cells of cardia, antrum, and in duodenal brunner glands. It is expressed in peri / supranuclear area.

MUC 3

It is not normally expressed in gastric mucosa. It is expressed in adenocarcinoma of stomach. It is related to serosal invasion,lymph node metastasis. It acts to protect the the tumor cell from adverse physiochemical condition such as low pH and involved in cellular adhesion. Its expression has poor prognosis.

IN NEOPLASTIC TRANSFORMATION

In atrophic gastritis

MUC 5AC and MUC 6 is expressed in columnar cells

In incomplete intestinal metaplasia

Increased expression of MUC 2 and MUC 3. Decreased expression of MUC5AC and MUC 6 in goblet cells and columnar cells.

In dysplasia

Decreased expression of MUC5AC and MUC 6 than intestinal metaplasia.

IN GASTRIC ADENOCARCINOMA

Early gastric cancer

There is a small expression of MUC 5 and MUC 6. Its expression is decreased in advanced cancerous stage.

In gastric type

Increased expression of MUC 5AC and MUC 6 in poorly differentiated carcinoma and signet ring cell carcinoma. They have increased expression of MUC 3 and

decreased expression of MUC 2.

In intestinal type

There is expression of MUC 2 and CD 10

Unclassified type

All MUC proteins are negative in this type.

Mucinous adenocarcinoma

There is increased expression of MUC 2. Expression of multiple mucin core peptides in gastric carcinoma is associated strongly with increased tumor stage. Increased multiple mucin expression reflect increased dedifferentiation and genetic alteration found in advanced carcinoma. It also contribute to tumor cell growth and metastatic abilities[20,18].

ON THE BASIS OF MUCIN HISTOCHEMISTRY[59]

Gastric cancer has been classified into

TYPE I - Gastric type **[G type]** - MUC 5AC and MUC 6 positive
MUC 2 and CD10 negative

TYPE II - Intestinal type **[I type]** - MUC 2 and CD 10 positive
MUC 5AC and MUC 6 negative

TYPE III - Gastrointestinal type **[GI]** - mixed type

TYPE IV - Null type **[N]**

TYPE II [Intestinal] is more common than other types

Table showing Master chart with subjective assessment of relative proportion of acid mucin and Neutral mucin in gastrectomy cases.

S.NO	HPE NO	AGE	SEX	REPORT	NEUTRAL MUCIN	ACID MUCIN
1.	3393/08	48	M	Moderately differentiated adenocarcinoma	30%	70%
2.	3409/08	44	F	Moderately differentiated adenocarcinoma	20%	80%
3.	3428/08	35	F	Moderately differentiated adenocarcinoma	40%	60%
4.	116/09	35	M	Poorly differentiated adenocarcinoma	10%	90%
5.	438/09	50	F	Moderately differentiated adenocarcinoma	80%	20%
6.	498/09	60	M	Moderately differentiated adenocarcinoma	50%	50%
7.	655/09	49	F	Poorly differentiated adenocarcinoma	10%	90%
8.	780/09	29	F	Poorly differentiated adenocarcinoma	10%	90%
9.	932/09	57	F	Well differentiated Adenocarcinoma with neuroendocrine differentiation	50%	50%
10.	2030/09	60	M	Mucinous adenocarcinoma	20%	80%
11.	2060/09	55	M	Early invasive adenocarcinoma stomach	60%	40%
12.	2195/09	40	M	Well differentiated Adenocarcinoma	20%	80%
13.	2472/09	64	M	Well differentiated Adenocarcinoma	10%	90%
14.	2783/09	48	M	Poorly differentiated adenocarcinoma	20%	80%
15.	3277/09	55	F	Well differentiated Adenocarcinoma	50%	50%
16.	3442/09	70	M	Moderately differentiated adenocarcinoma	10%	90%

S.NO	HPE NO	AGE	SEX	REPORT	NEUTRAL MUCIN	ACID MUCIN
17.	3515/09	55	M	Moderately differentiated adenocarcinoma	30%	70%
18.	950/10	55	M	Moderately differentiated adenocarcinoma	30%	70%
19.	1377/10	51	F	Mucinous adenocarcinoma	10%	90%
20.	1982/10	65	M	Poorly differentiated adenocarcinoma	20%	80%
21.	2198/10	66	M	Moderately differentiated adenocarcinoma	30%	70%
22.	2308/10	55	M	Poorly differentiated adenocarcinoma	10%	90%
23.	2405/10	55	M	Moderately differentiated adenocarcinoma	30%	70%
24.	2433/10	50	F	Moderately differentiated adenocarcinoma	30%	70%
25.	2834/10	60	M	Moderately differentiated adenocarcinoma	40%	60%
26.	2951/10	60	F	Squamous cell carcinoma	-	
27.	3157/10	52	M	Poorly differentiated adenocarcinoma	10%	90%
28.	3217/10	80	M	Moderately differentiated adenocarcinoma	10%	90%
29.	3355/10	48	M	Moderately differentiated adenocarcinoma	50%	50%
30.	3441/10	50	F	Moderately differentiated adenocarcinoma	60%	40%
31.	3583/10	47	M	Signet ring cell carcinoma	PAS - Neutral mucin	
32.	3735/10	25	F	Poorly differentiated adenocarcinoma	30%	70%
33.	3737/10	65	F	Poorly differentiated adenocarcinoma	20%	80%
34.	4059/10	50	F	Poorly differentiated adenocarcinoma	30%	70%
35.	4335/10	58	M	Moderately differentiated adenocarcinoma	30%	70%

S.NO	HPE NO	AGE	SEX	REPORT	NEUTRAL MUCIN	ACID MUCIN
36.	4401/10	53	F	Poorly differentiated adenocarcinoma	10%	90%
37.	51/11	60	M	Poorly differentiated adenocarcinoma	30%	70%
38.	579/11	40	F	Poorly differentiated adenocarcinoma	10%	90%
39.	852/11	40	F	Moderately differentiated adenocarcinoma	30%	70%
40.	863/11	66	F	Moderately differentiated adenocarcinoma	40%	60%
41.	964/11	50	M	Poorly differentiated adenocarcinoma	10%	90%
42.	1121/11	61	F	Moderately differentiated adenocarcinoma	40%	60%
43.	1162/11	65	M	Moderately differentiated adenocarcinoma	10%	90%
44.	1175/11	60	F	Poorly differentiated adenocarcinoma	20%	80%
45.	1287/11	64	M	Well differentiated Adenocarcinoma	20%	80%
46.	1537/11	55	M	Moderately differentiated adenocarcinoma	10%	90%
47.	1826/11	50	M	Well differentiated Adenocarcinoma	10%	90%
48.	2160/11	58	M	Moderately differentiated adenocarcinoma	30%	70%
49.	2876/11	35	M	Poorly differentiated adenocarcinoma	10%	90%
50.	3129/11	48	F	Poorly differentiated adenocarcinoma	10%	90%

The mucin was predominantly acidic.

OBSERVATION AND RESULTS

During the period October 2008 to September 2011, a total of 13,593 cases were received, of which 303 cases were from gastric biopsies and 50 cases were gastrectomy specimens .

Table 1 ; Gastric endoscopic biopsies results of male

	CHRONIC GASTRITIS				INTESTINAL METAPLASIA				DYSPLASIA				CARCINOMA				NORMAL				NIL TISSUE			
AGE	08 Oct	09	10	11 sep	08 oct	09	10	11 sep	08 oct	09	10	11 sep	08 oct	09	10	11 sep	08 oct	09	10	11 sep	08 oct	09	10	11 sep
21-30	-	-	1	3	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
31-40	-	-	5	1	-	-	-	-	-	1	-	-	2	3	1	1	-	1	-	-	-	1	-	1
41-50	2	7	7	6	-	1	-	-	1	5	-	-	2	16	8	3	-	2	-	-	-	2	1	2
51-60	-	8	10	5	-	1	1	-	-	4	2	3	2	14	12	6	-	-	-	-	-	4	2	3
61-70	3	5	6	4	-	1	-	-	-	1	1	1	1	12	14	5	-	-	-	-	-	-	1	-
71-80	-	2	2	-	-	-	-	-	-	-	-	-	-	3	2	1	-	-	-	-	-	-	-	-
>80	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
?AGE	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-

Chart :1

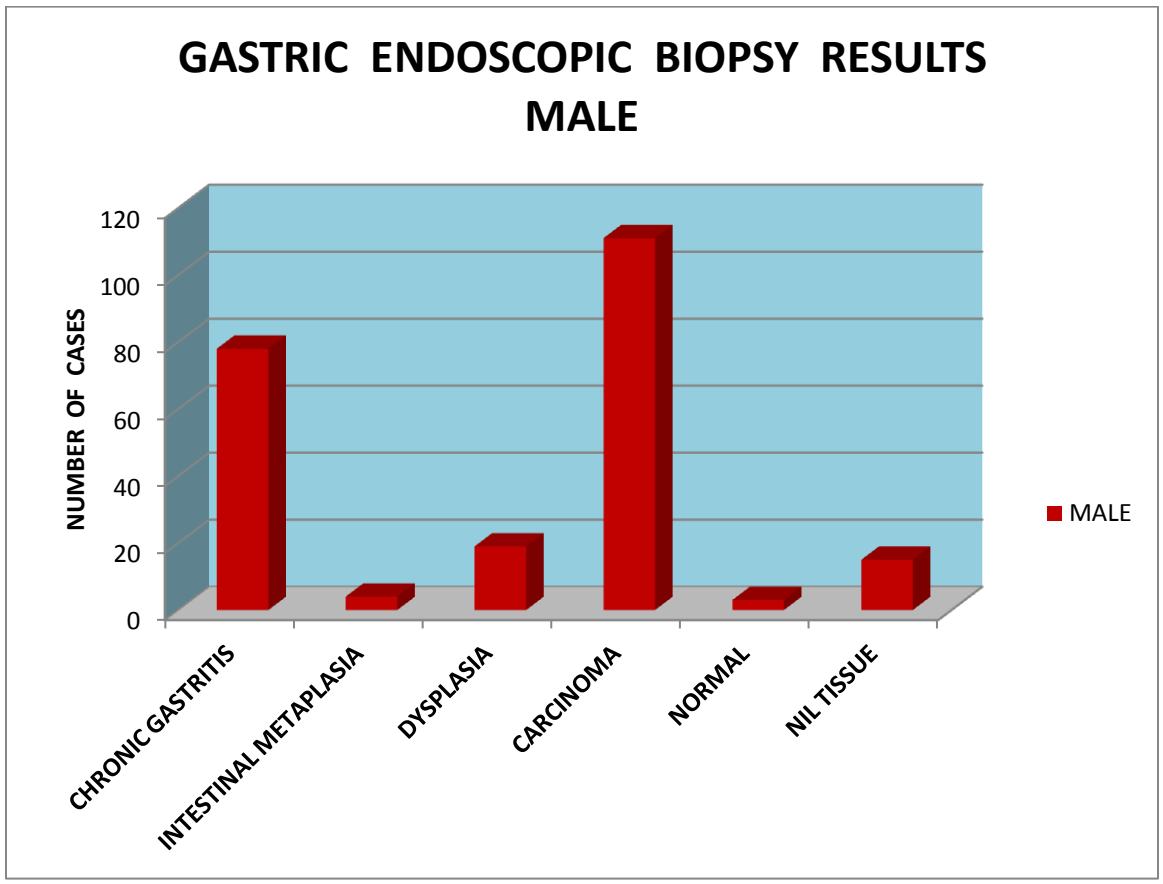


Table 2; From gastric endoscopic biopsies, incidence of gastric cancer in male

Total endoscopic biopsies	Male cases	Gastric cancer
303	230	111

Among the biopsies in males, most of them were carcinoma 111 (48.2%), it was around 36.6% in total gastric endosopic biopsies. The maximum incidence occurred in the 6th decade (30.6%) followed by 7th decade (28.8%) and 5th decade (26.1%).

Next to carcinoma, most of them were chronic gastritis , followed by dysplasia.

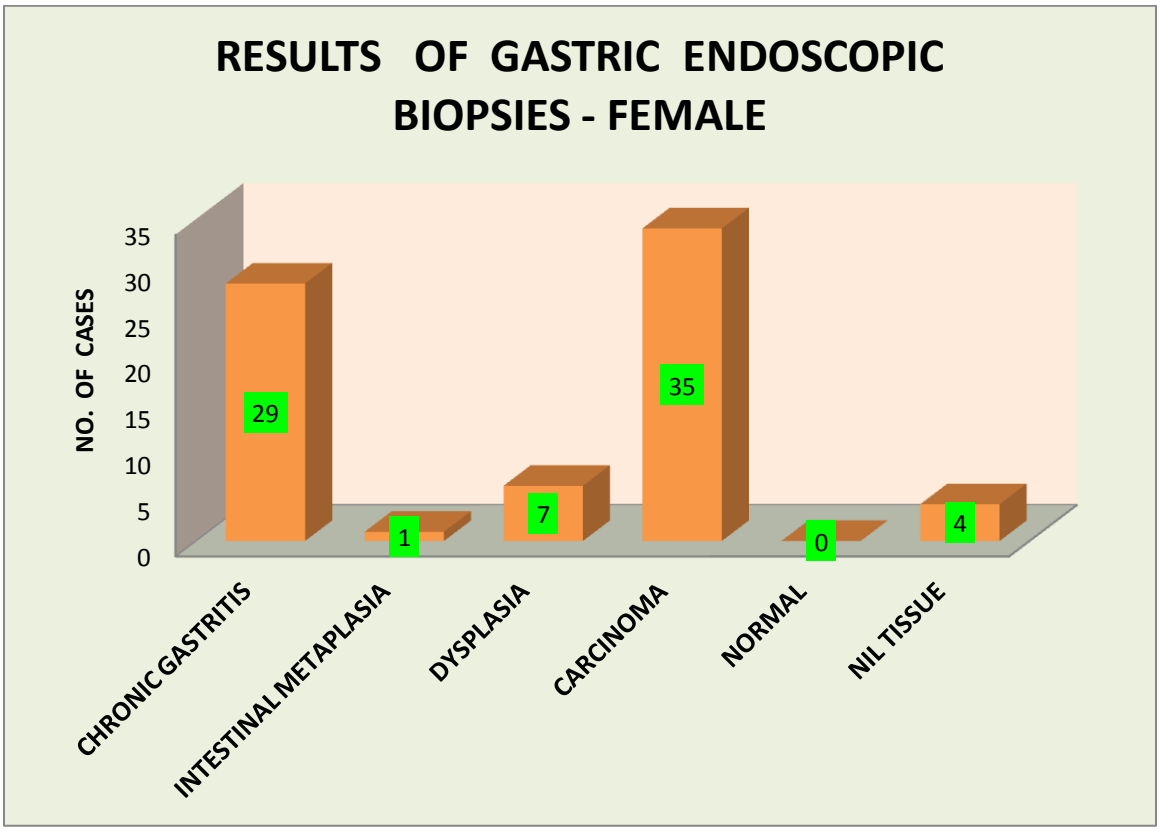
Table 3; Endoscopic results of female –gastric endoscopic biopsy from 2008 oct - 2011 sep

	CHRONIC GASTRITIS				INTESTINAL METAPLASIA				DYSPLASIA				CARCINOMA				NORMAL				NIL TISSUE			
AGE	08	09	10	11	08	09	10	11	08	09	10	11	08	09	10	11	08	09	10	11	08	09	10	11
21-30	-	2	4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
31-40	-	1	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41-50	-	3	4	-	-	-	-	-	1	3	-	-	2	4	5		-	-	-	-	-	2	-	-
51-60	1	2	5	-	-	-	1	-	-	1	-	-	-	5	4	2	-	-	-	-	-	1	-	-
61-70	-	-	1	1	-	-	-	-	-	1	1	-	-	5	1	2	-	-	-	-	-	1	-	-
71 -80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
?AGE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4; From gastric endoscopic biopsies, incidence of gastric cancer in female

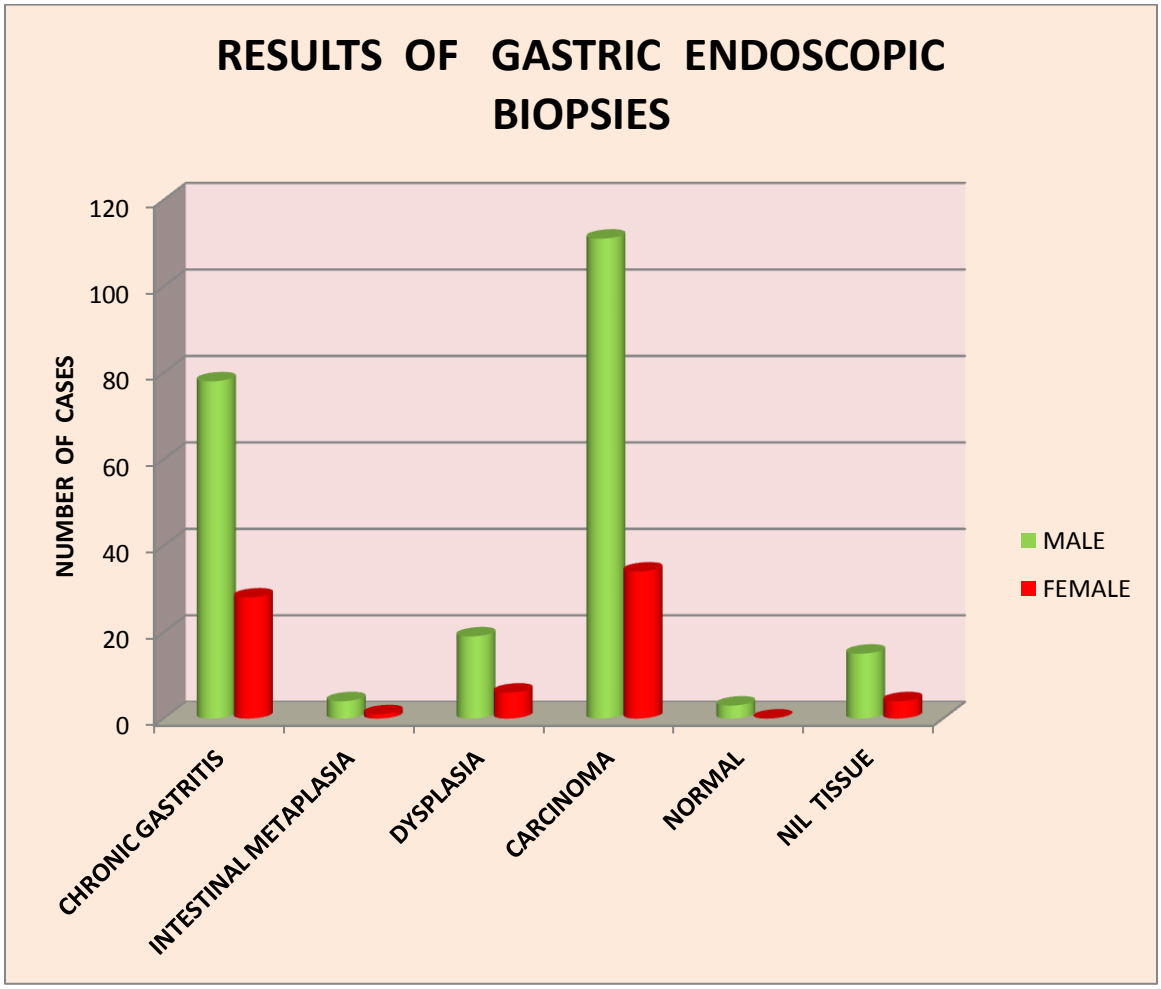
Total gastric endoscopic biopsies	Female cases	Gastric cancer
303	73	34

Chart: 2



Of the total gastric biopsies received for female, most of the cases were gastric carcinoma 34 in 73 cases(46.6%) followed by chronic gastritis and dysplasia. The maximum incidence of gastric carcinoma occurred in 6^{th} and 5^{th} decade (32.3%) followed by 7^{th} decade .(23.5%)

Chart: 3



The incidence of gastric carcinoma and dysplasia was more common in male with ratio of 3:1, followed by chronic gastritis.

In Thanjavur Medical College , during the period October 2008 - September 2011, a total of 13,593 specimens were received. It include 303 gastric biopsies and 50 gastrectomy specimens.

A total of 353 gastric specimens were received during this period, of which 195 (55.25%) cases were reported as gastric cancer.

Chart:4

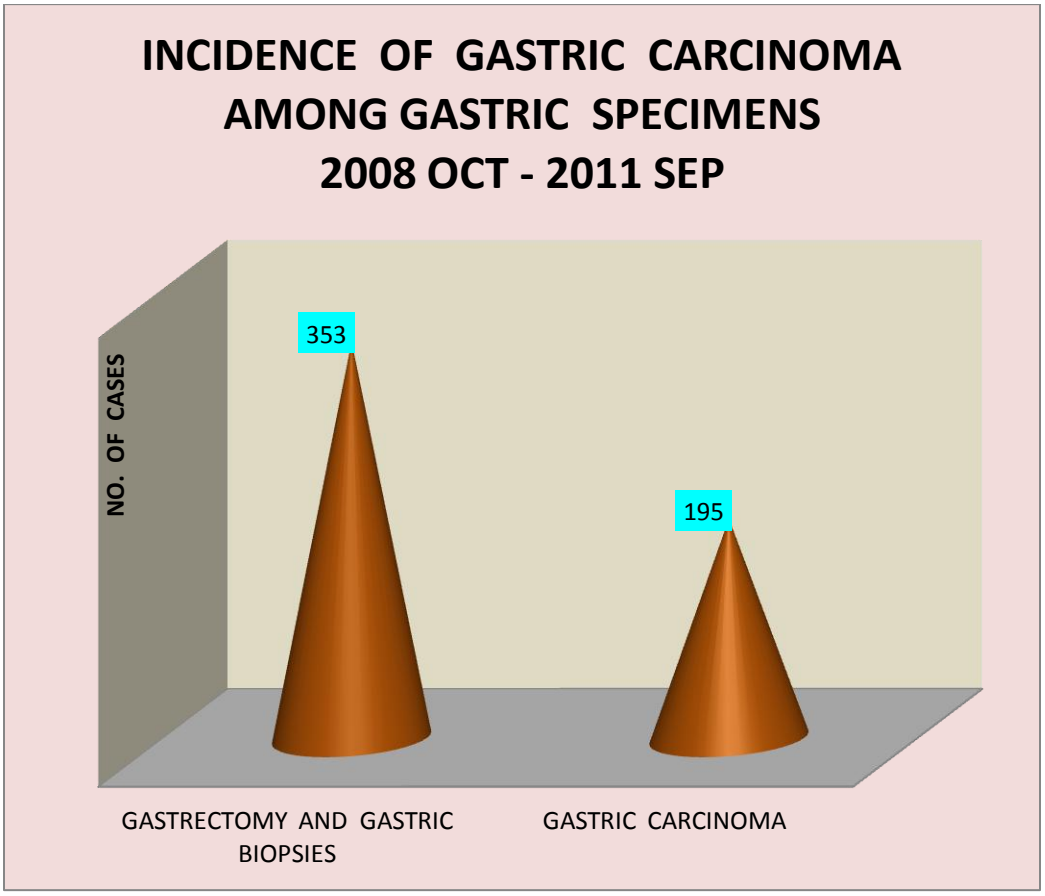
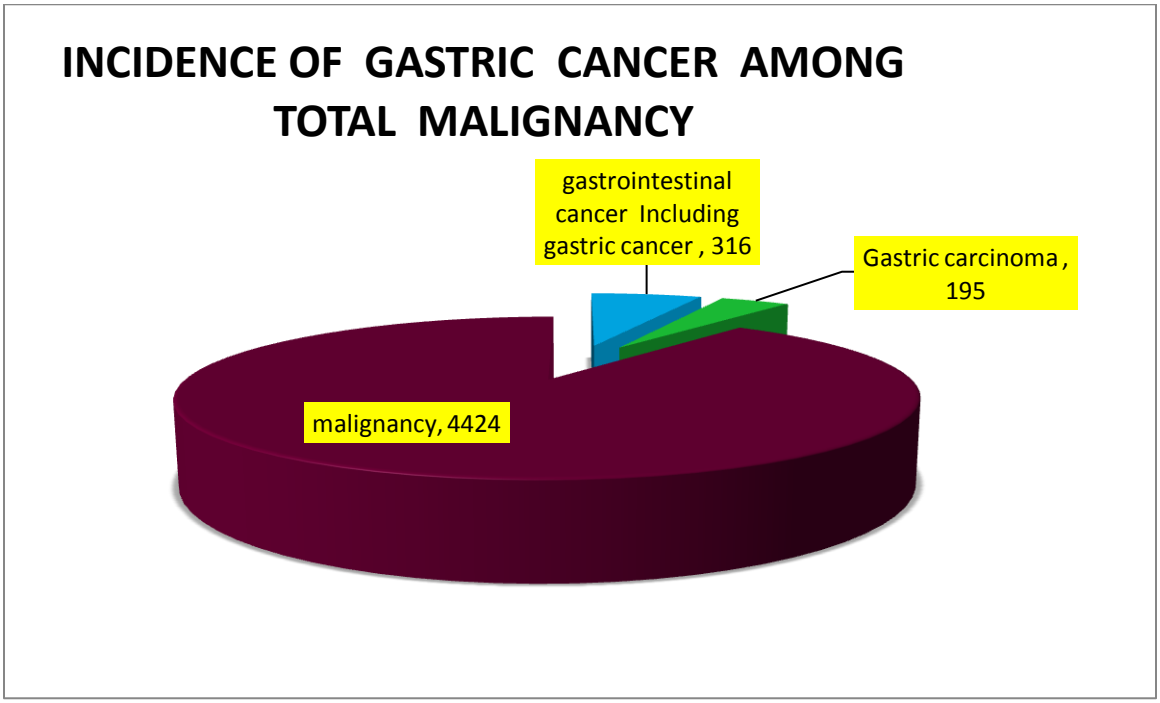


Table 5; incidence of gastric cancer among received specimens.

Period of study	Total cases	No. of malignancy	Gastric cancer
2008 oct – 2011 sep	13,593	4424	195
Percentage of cases		32.5 % in received Cases	4.4% In overall cancers

Chart:5

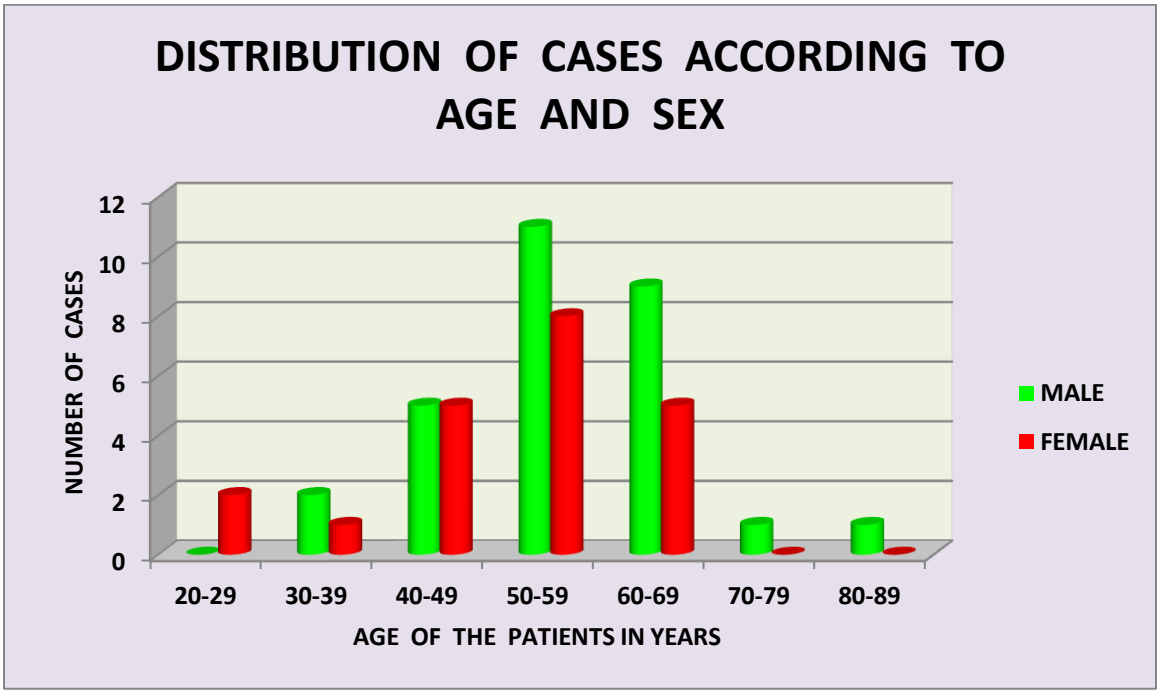


Among 13,593 cases received , 4424 (32.5%) cases were reported as malignancy. Of overall malignancy, 316 (7.14%) cases were gastrointestinal cancer ,of them 195 (61.70%) cases were gastric cancer.

Table 6; The distribution of cases according to age and sex is shown

AGE	MALE	FEMALE
20-29	-	2
30-39	2	1
40-49	5	5
50-59	11	8
60-69	9	5
70-79	1	-
80-89	1	-

Chart :6



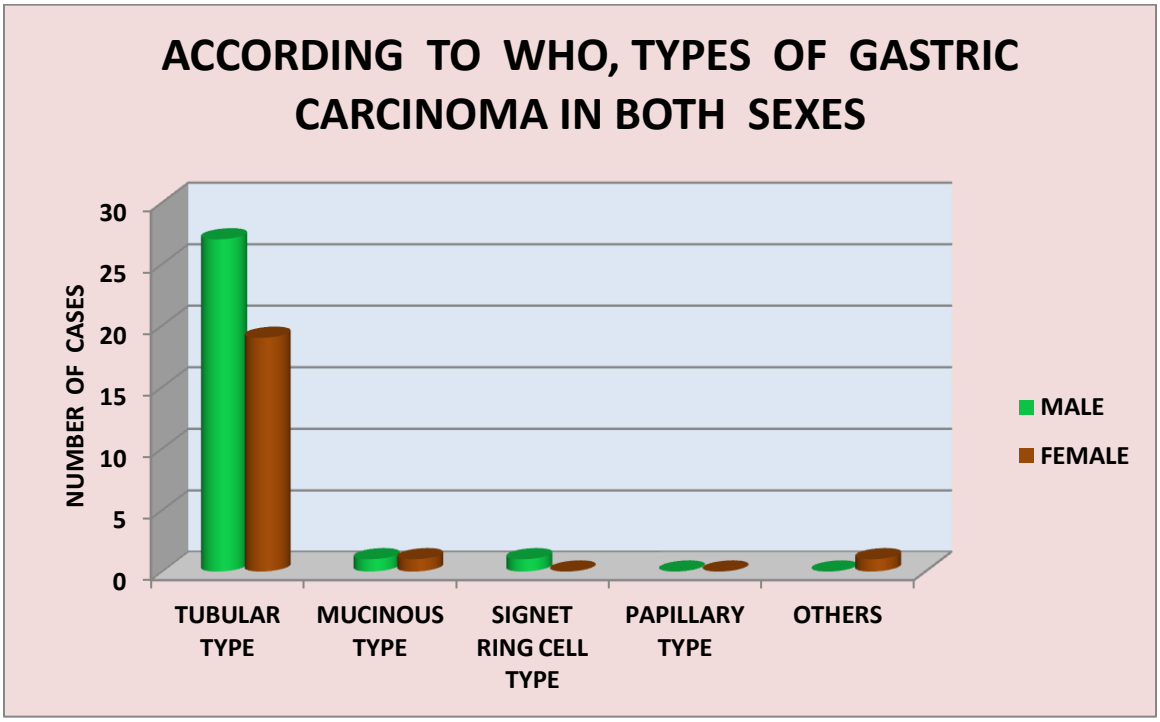
Out of 50 cases, 29 cases were from male and 21 cases were from female, maximum number of cases were seen in 6th decade for male patients and female patients.

HISTOLOGICAL TYPING OF TUMOR

Table 7; According to WHO classification

TYPES OF GASTRIC CARCINOMA	MALE	FEMALE
TUBULAR	27	19
MUCINOUS	1	1
SIGNET RING CELL	1	-
PAPILLARY	-	-
OTHERS	-	1

Chart :7

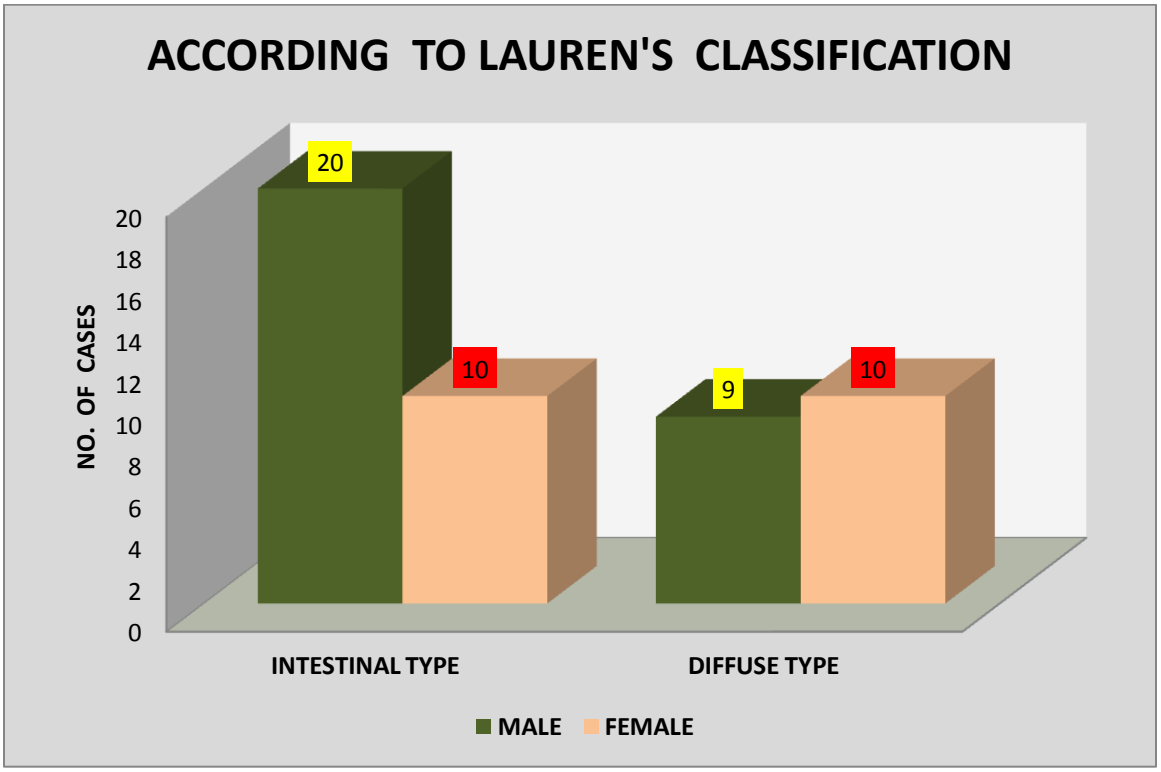


Most of the tumors were tubular carcinoma around 92%. Pure signet ring cell carcinoma fig ( ) was around 2% and mucinous fig ( )was around 4%. Squamous cell carcinoma was around 2% .

Table 8 ; According to Lauren's classification

TYPES OF GASTRIC CANCER	MALE	FEMALE
INTESTINAL	20	10
DIFFUSE	9	10

Chart :8



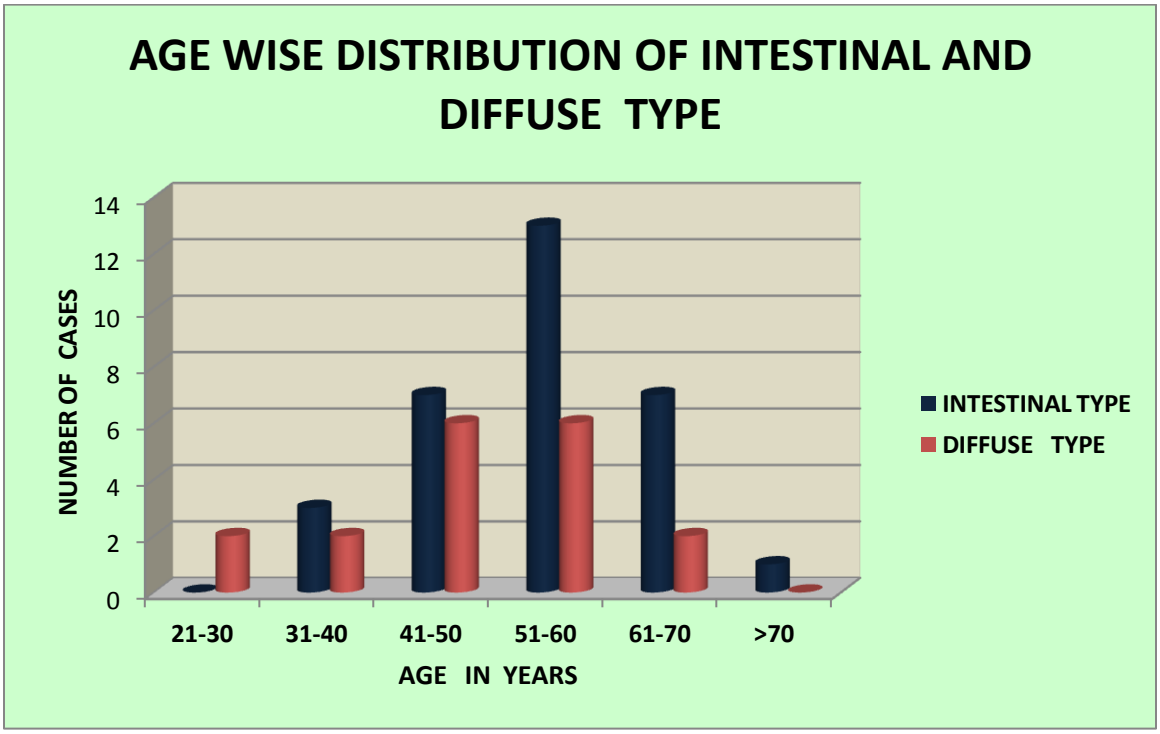
Among 50 specimens, 49 cases were adenocarcinoma. Of which, 62% cases were intestinal type, 39% were of diffuse type. The intestinal type showed male predominance, the diffuse type was equal in both sexes.

TABLE 9 ;AGE WISE DISTRIBUTION OF GASTRIC TUMOR

[LAUREN'S CLASSIFICATION]

AGE GROUP	INTESTINAL TYPE	DIFFUSE TYPE
21 - 30	-	2
31 - 40	3	3
41 - 50	7	6
51 - 60	12	7
61 - 70	7	1
>7O	1	-

Chart: 9



In contrast to intestinal type, diffuse type is more common in young age with equal incidence in both high risk and low risk geographic areas due to regulation by genetic factors

Table 10 ; According to Japanese society for gastric carcinoma,

TYPES OF GASTRIC CA	MALE	FEMALE
PAPILLARY	0	0
TUBULAR	20	10
POORLY DIFFERENTIATED	7	9
MUCINOUS	1	1
SIGNET RING CELL	1	0
OTHERS	0	1

Chart: 10

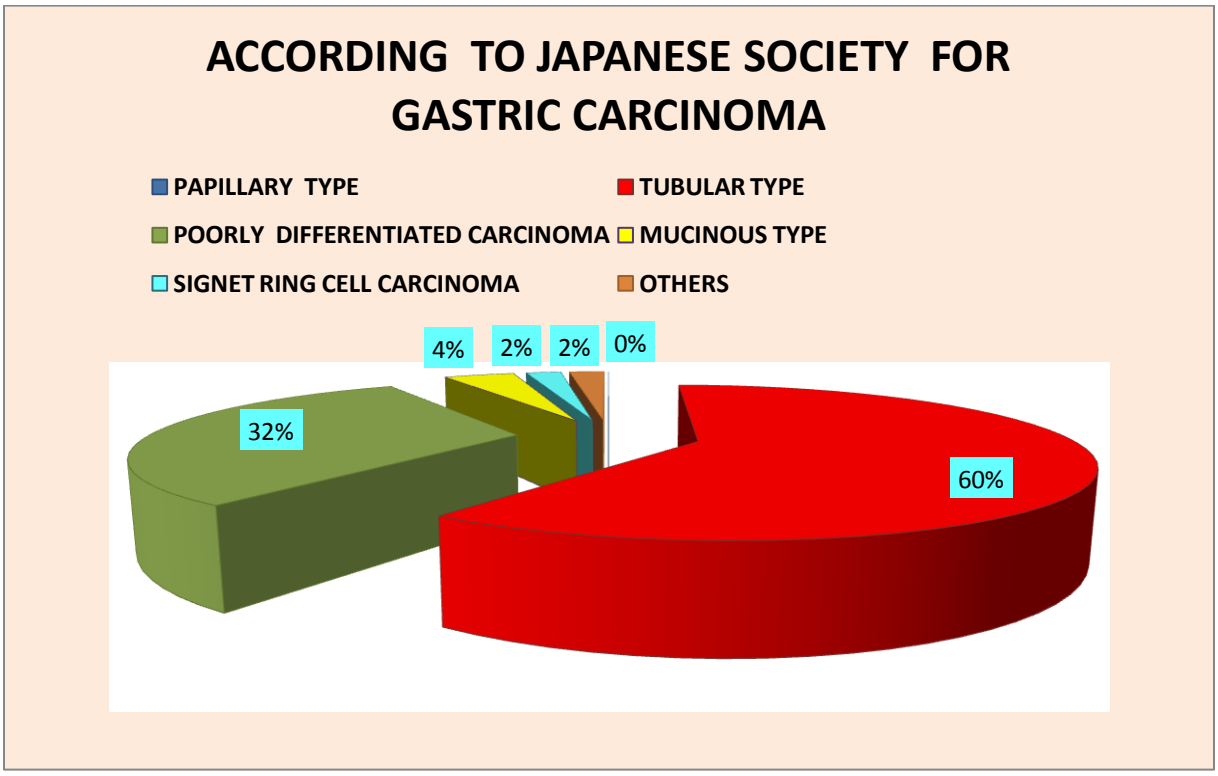


TABLE 11: According to Nakamura's classification;

GRADING	MALE	FEMALE
UNDIFFERENTIATED	9	10
DIFFERENTIATED	20	10
OTHERS	-	1

Chart :11

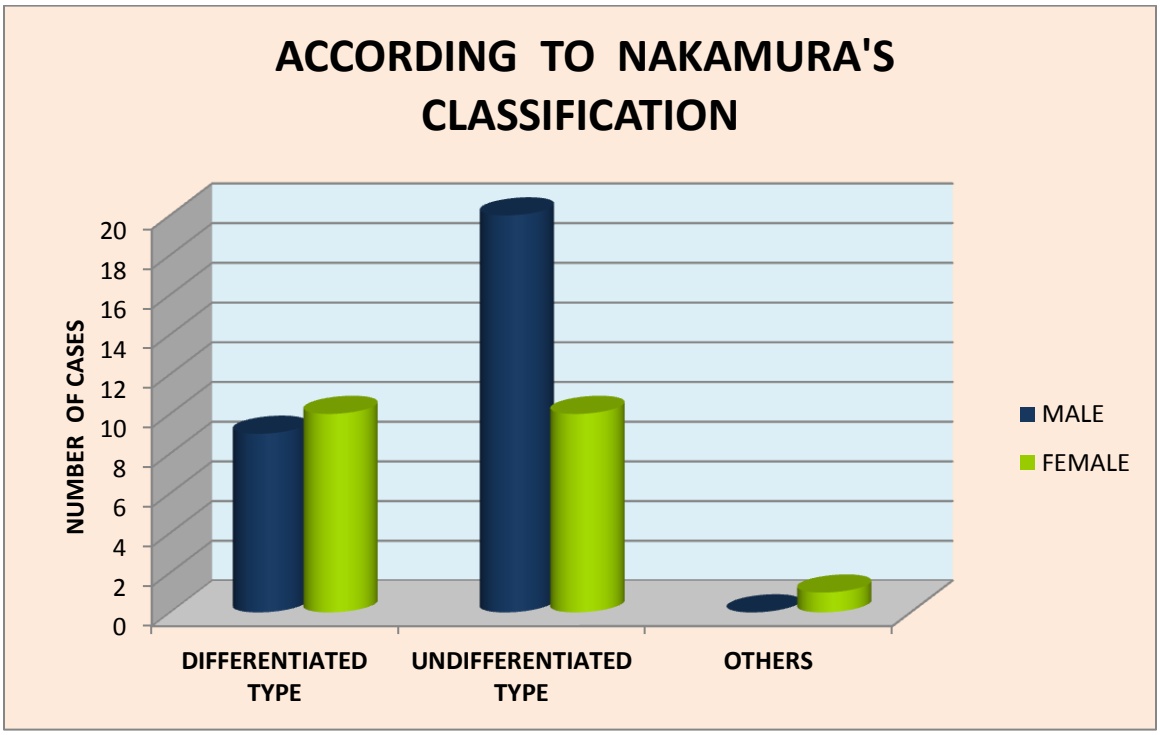
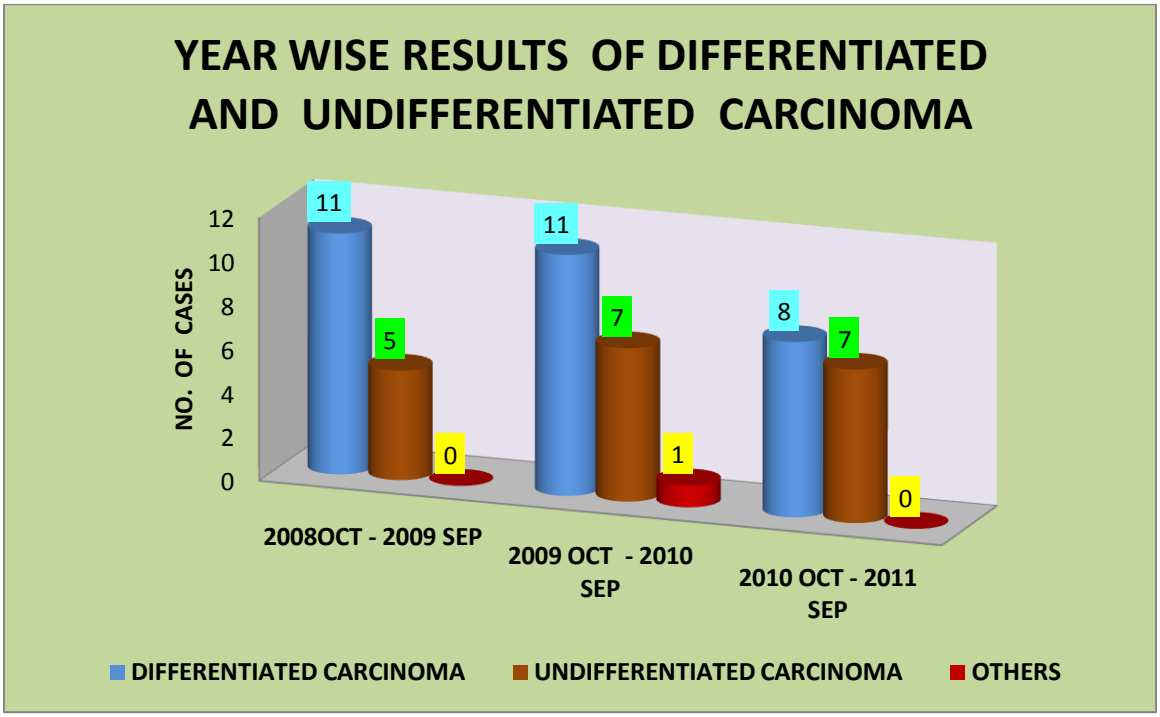


Table 12; year wise results of the differentiated and undifferentiated carcinoma;

YEAR	DIFFERENTIATED CARCINOMA	UNDIFFERNTIATED CARCINOMA	OTHERS
2008 OCT - 2009 SEP	11	5	-
2009 OCT – 2010 SEP	11	7	1
2010 OCT – 2011 SEP	8	7	-

Chart; 12



Of the 50 gastrectomy specimens, 49 cases were adenocarcinoma, one case was Squamous cell carcinoma. Table 12, shows most of them were differentiated adenocarcinoma

Table 13; Distribution of early and advanced gastric cancer

Types of cancer	Early cancer	Advanced cancer
No. of cancers	1	49

Early gastric cancer is invasive adenocarcinoma of stomach confined tothe mucosa or submucosa regardless of lymph node metastasis. In this study 98% of cases were advanced cancer fig (1,2,3) ,2% were early cancer.

Table 14; On the basis of differentiation,

Differentiation	Well differentiated Adenocarcinoma	Moderately differentiated adenocarcinoma	Poorly differentiated adenocarcinoma
No. of cases	7	22	17

Of 50 cases , 14.2% were well differentiated fig (8,9) ,44.8% were moderately differentiated, 34.7% were poorly differentiated adenocarcinoma. fig (10,11)

FIG 1 ;Ulcerative growth with heaped up margin in antropyloric region Measuring 6x4cm invading upto serosa

FIG 2 ; Ulcerative growth in the antropyloric region M 5x3 cm with adjacent ironed out mucosa and metastatic node (arrow)

FIG 3 ; Ulcerative growth in the antropyloric region M 6x3 cm with thickened wall and adjacent ironed out mucosa

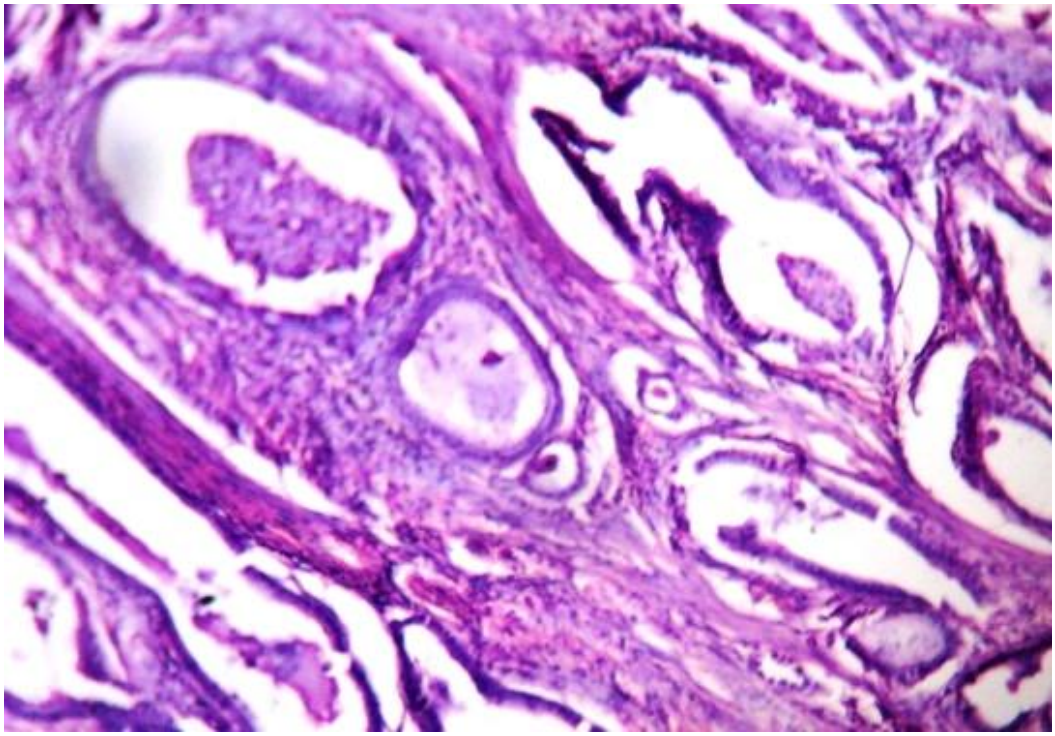


FIG 8 ; Well differentiated adenocarcinoma showing tubular glands invading into Muscularis propria

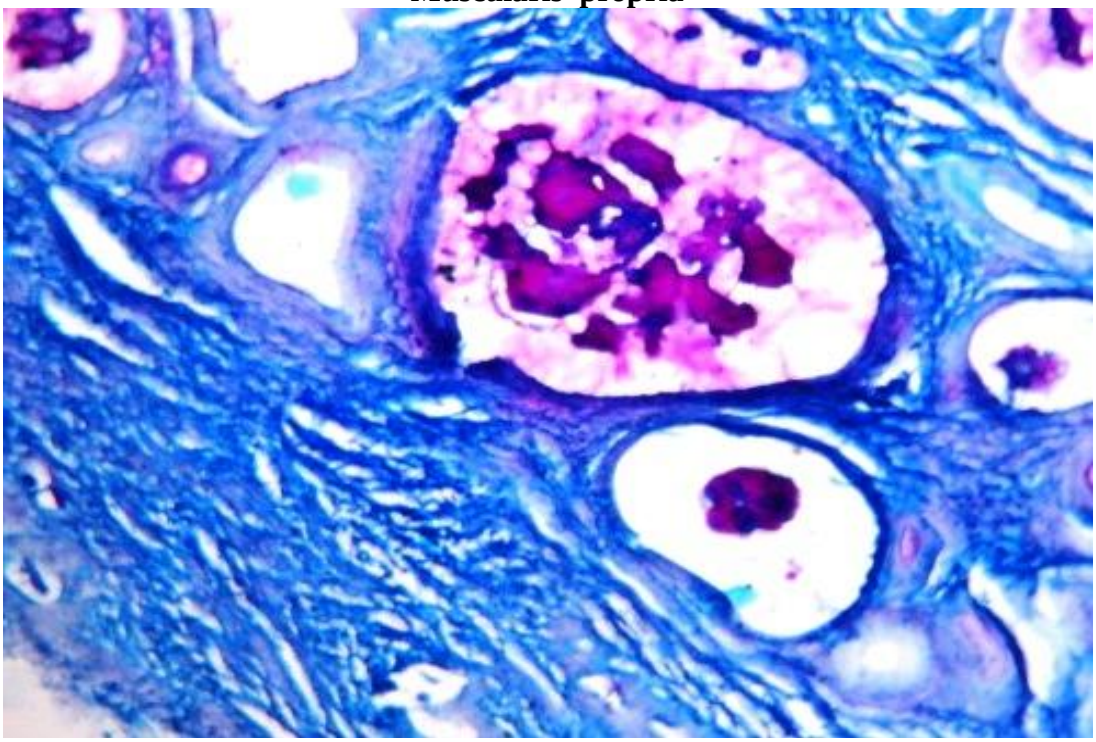


FIG 9 ; Well differentiated adenocarcinoma stained with AB pH 2.5 PAS showing tubular glands filled with neutral mucin in magenta color (X 400)

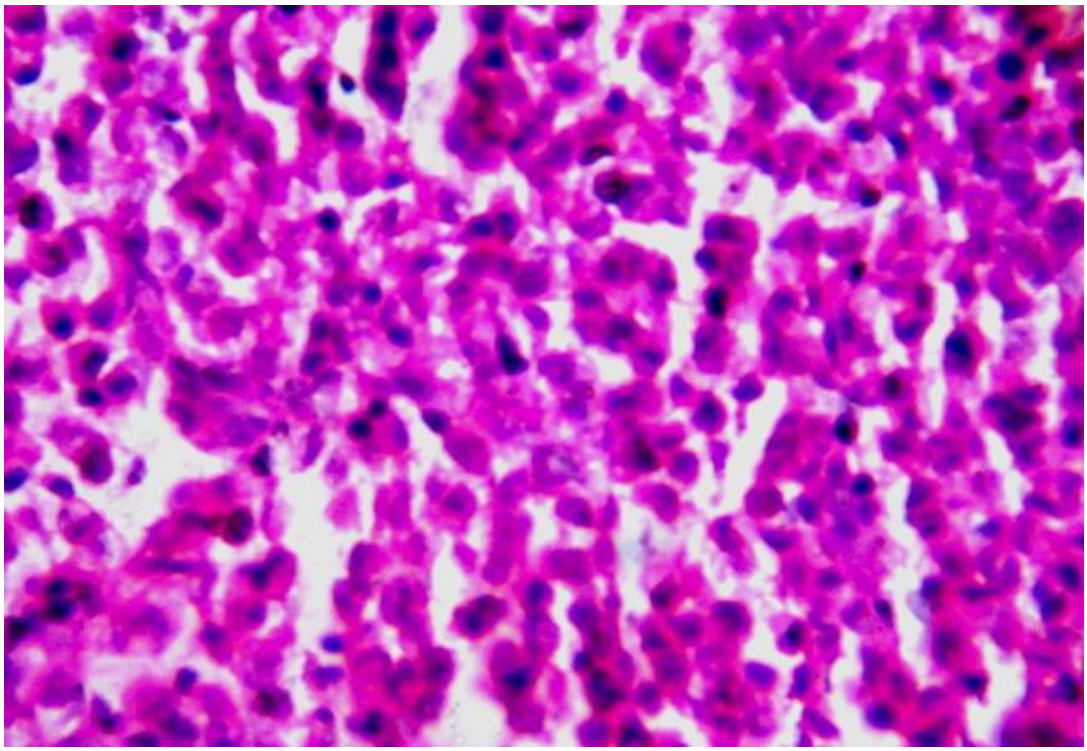


FIG 10 ; Poorly differentiated adenocarcinoma with malignant cells in diffuse pattern H & E X 400

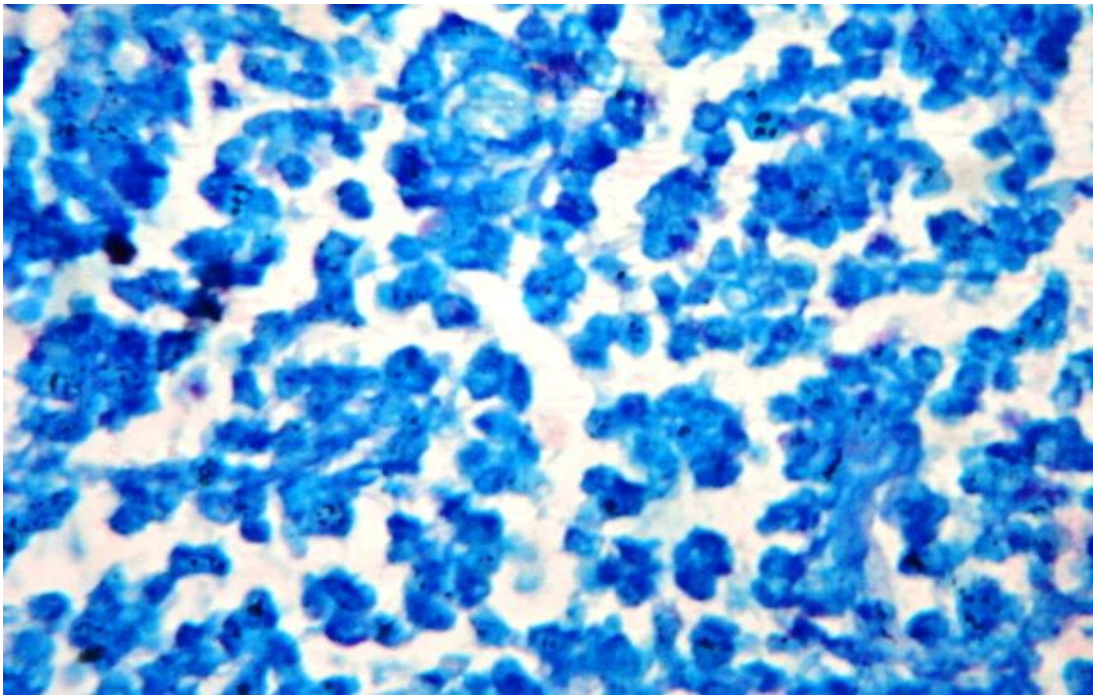


FIG 11 ; Poorly differentiated adenocarcinoma in AB pH 2.5 PAS showing malignant cells with acid mucin in blue color X 400

SPECIAL STAIN STUDY

Gastrectomy cases were evaluated for mucin histochemistry by using combined Alcian Blue pH2.5 PAS and PAS [Periodic acid Schiff stain] .Acid mucin was expressed in 48 cases [96%] including intestinal metaplasia fig (4,5,6,7) , Mucinous adenocarcinoma fig(12,13,14)

signet ring cell carcinoma was stained by PAS shows neutral mucin expression.fig (15,16,17,18)

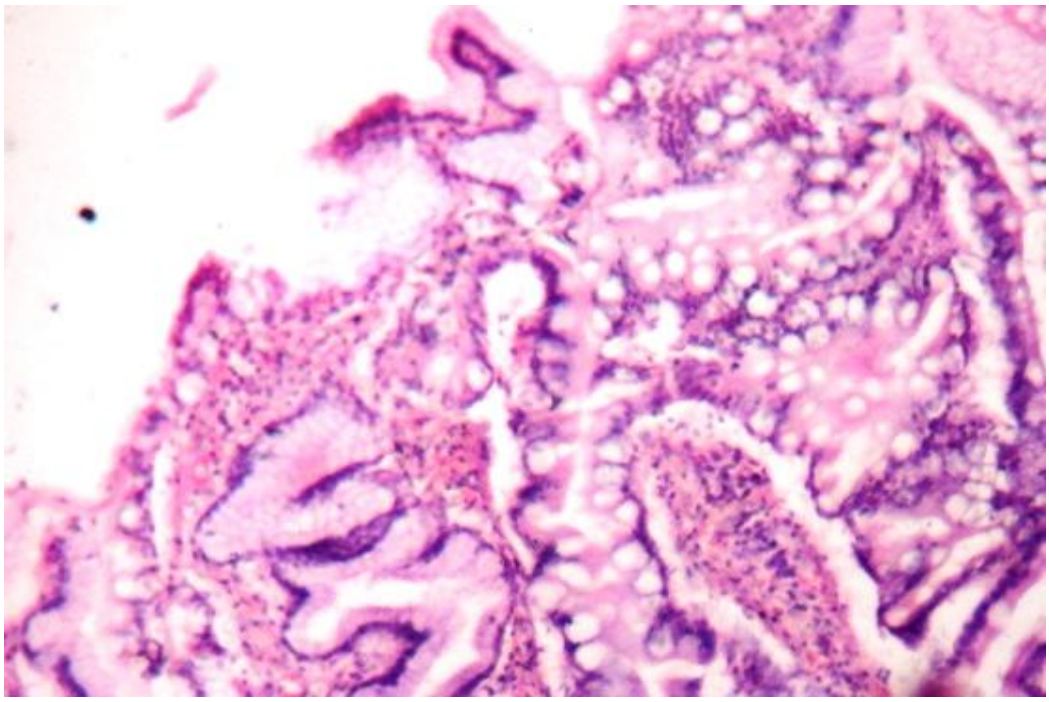


FIG 4 ; Intestinal metaplasia in gastric mucosa showing goblet cells along with columnar cells, H&E X 100

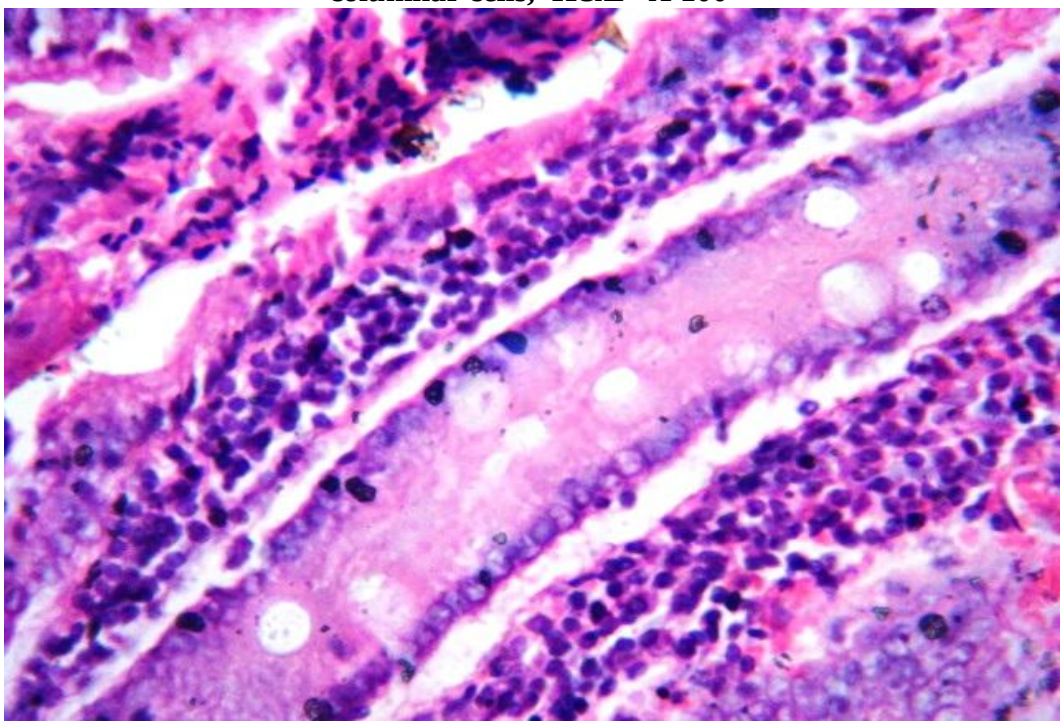


FIG 5 ; Intestinal metaplasia in gastric mucosa H&E X 400

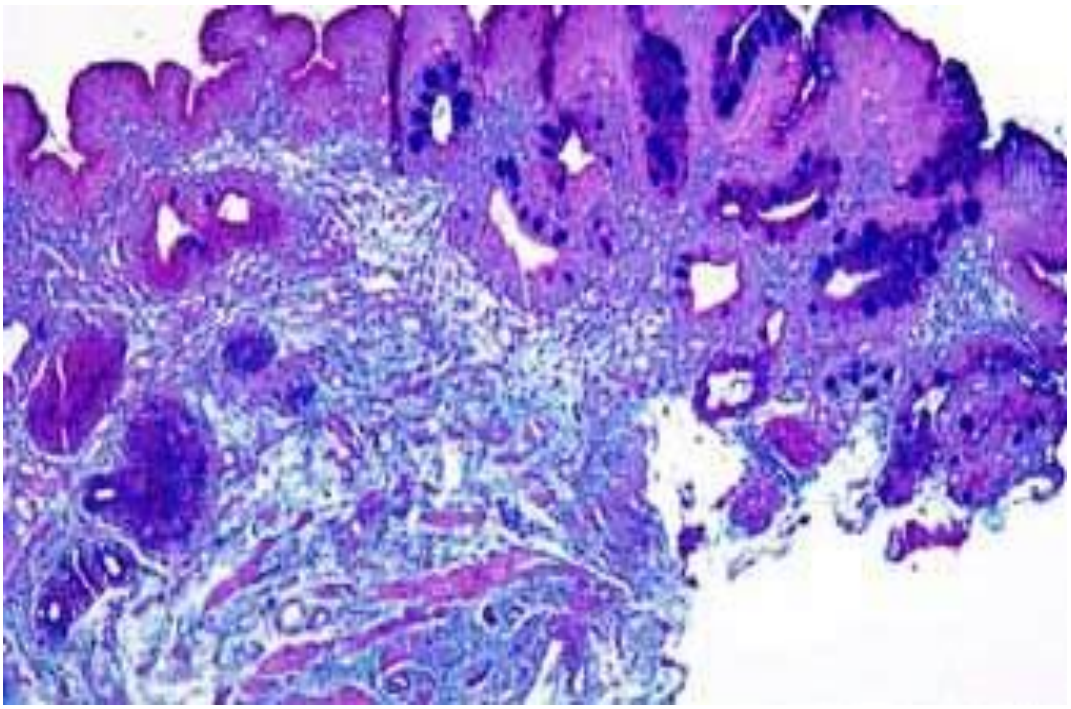


FIG 6 ; Intestinal metaplasia exhibited by AB pH 2.5 PAS (scanner view)

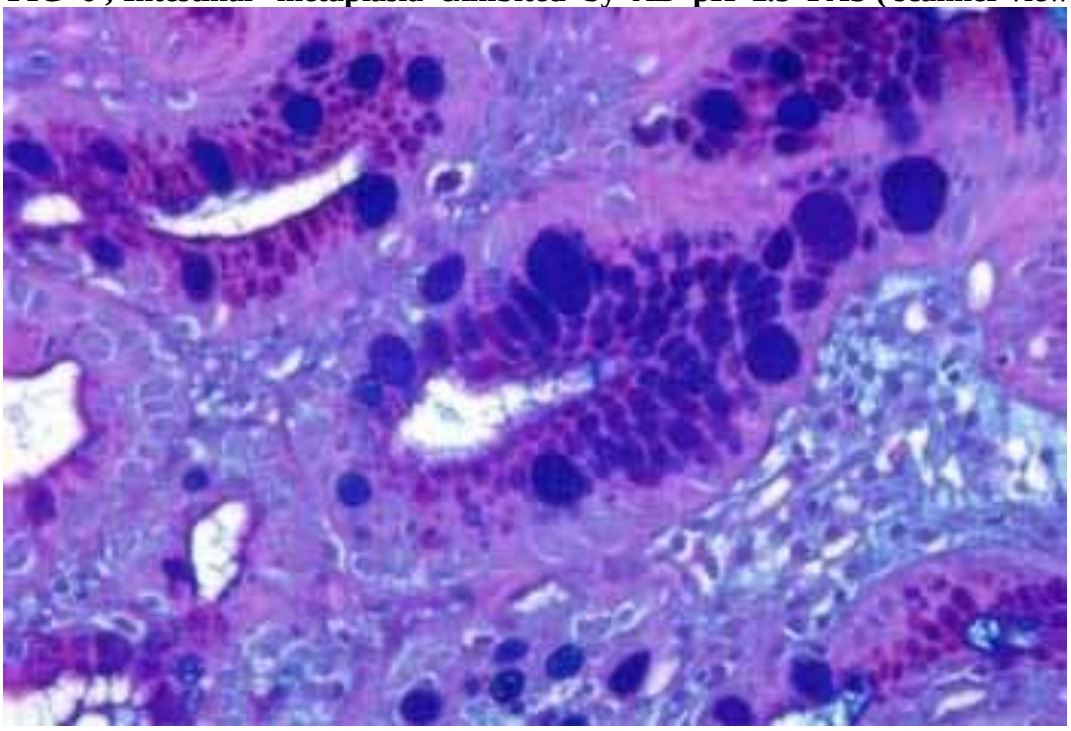


FIG 7; Intestinal metaplasia exhibited by AB pH 2.5 PAS showing goblet cells with acid mucin in blue color X400

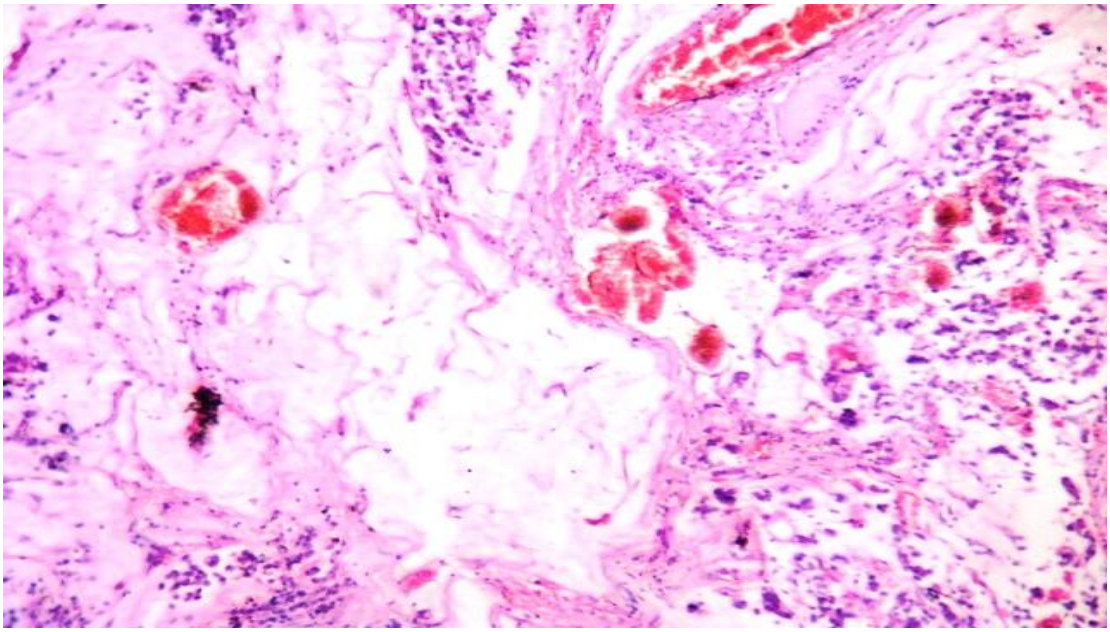


FIG 12 ; Mucinous adenocarcinoma showing malignant cells floating in mucinous pool. H&E X100

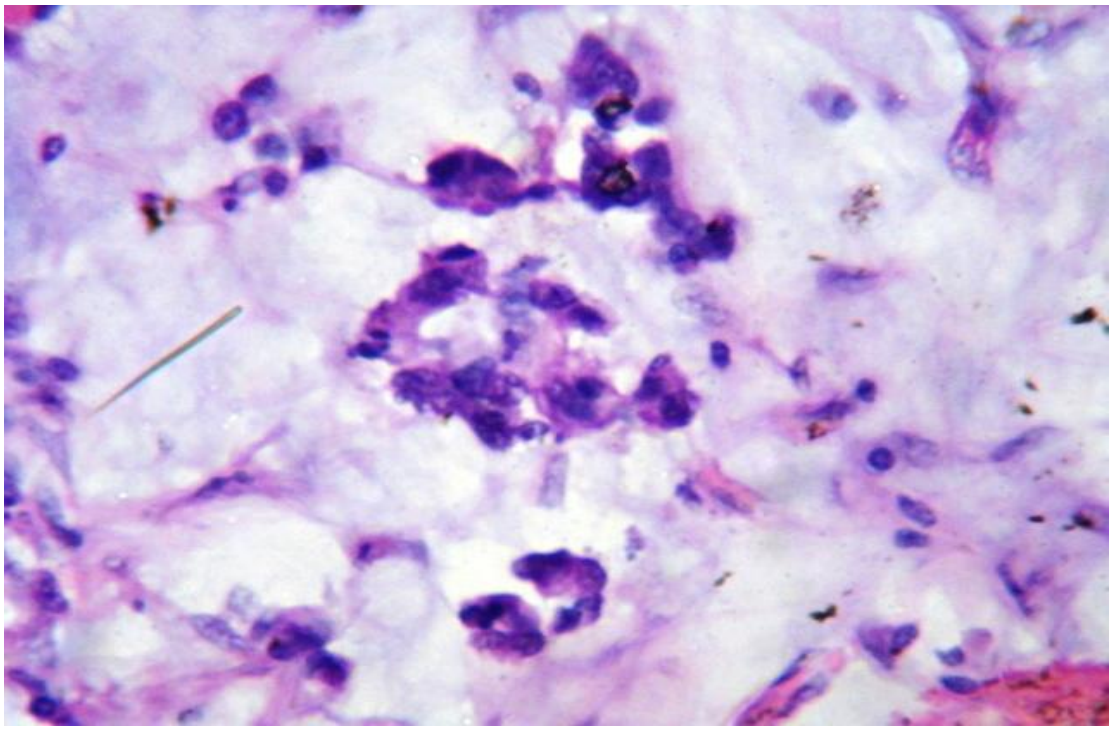


FIG 13 ; Mucinous adenocarcinoma showing malignant cells in mucinous pool H&E X400

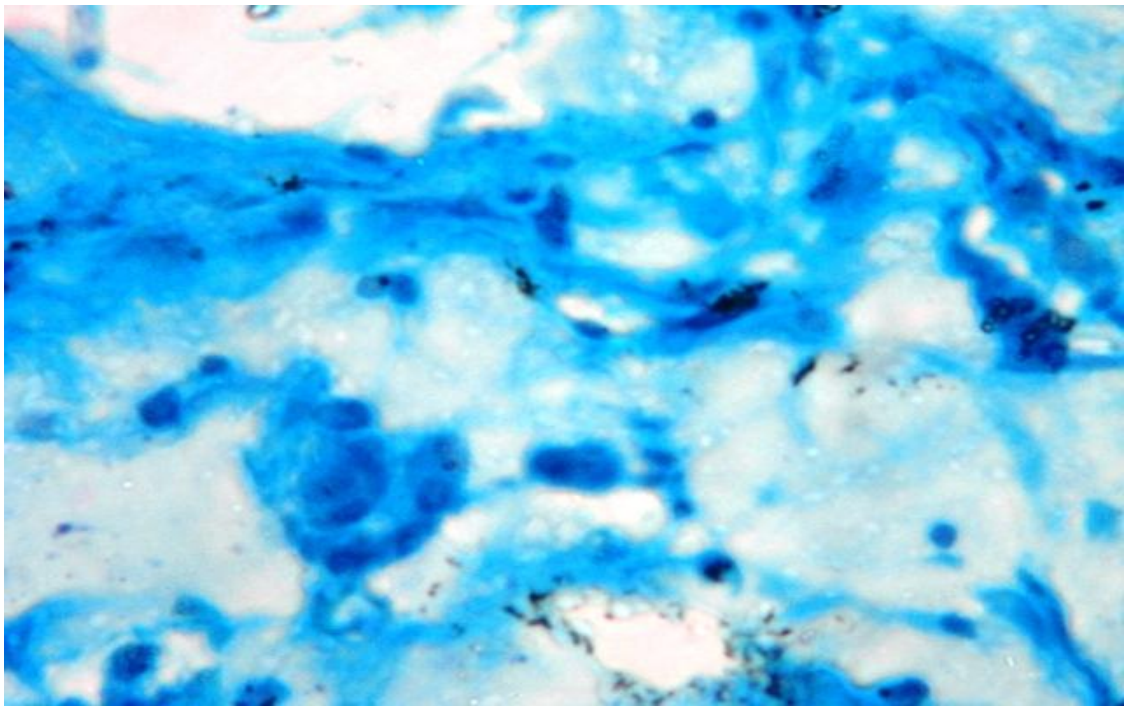


FIG 14 ; Mucinous adenocarcinoma showing malignant cells in mucinous pool in AB pH 2.5 PAS showing acid mucin in blue color X 400

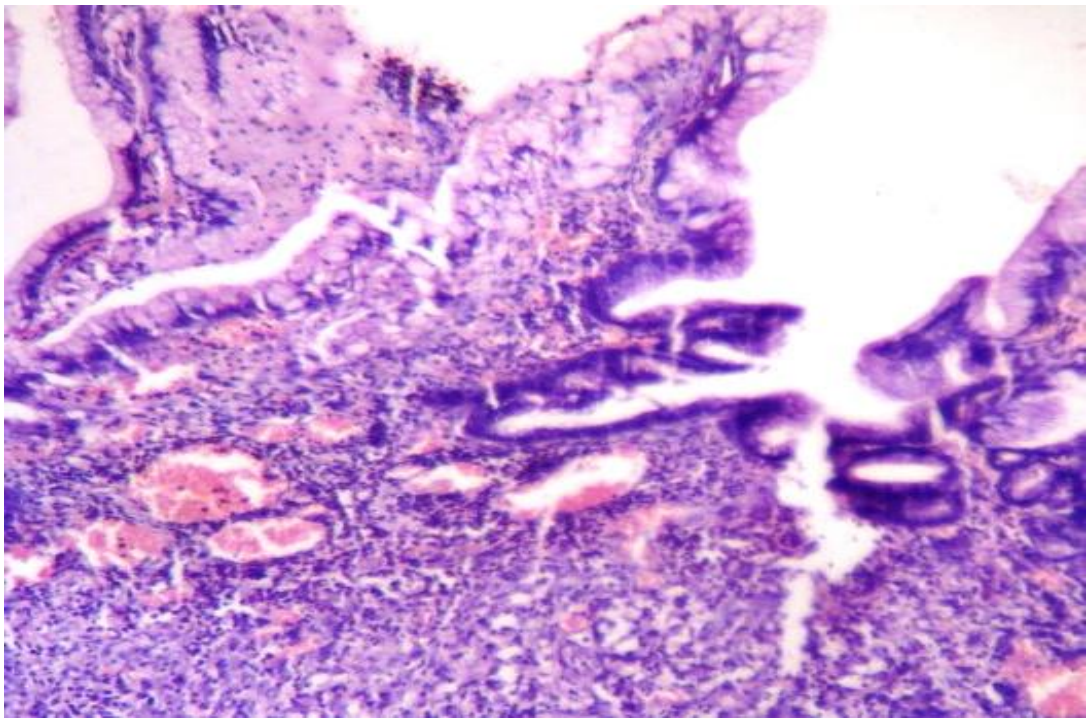


FIG 15; Signet ring cell carcinoma showing diffusely arranged signet ring cells H&E x 100

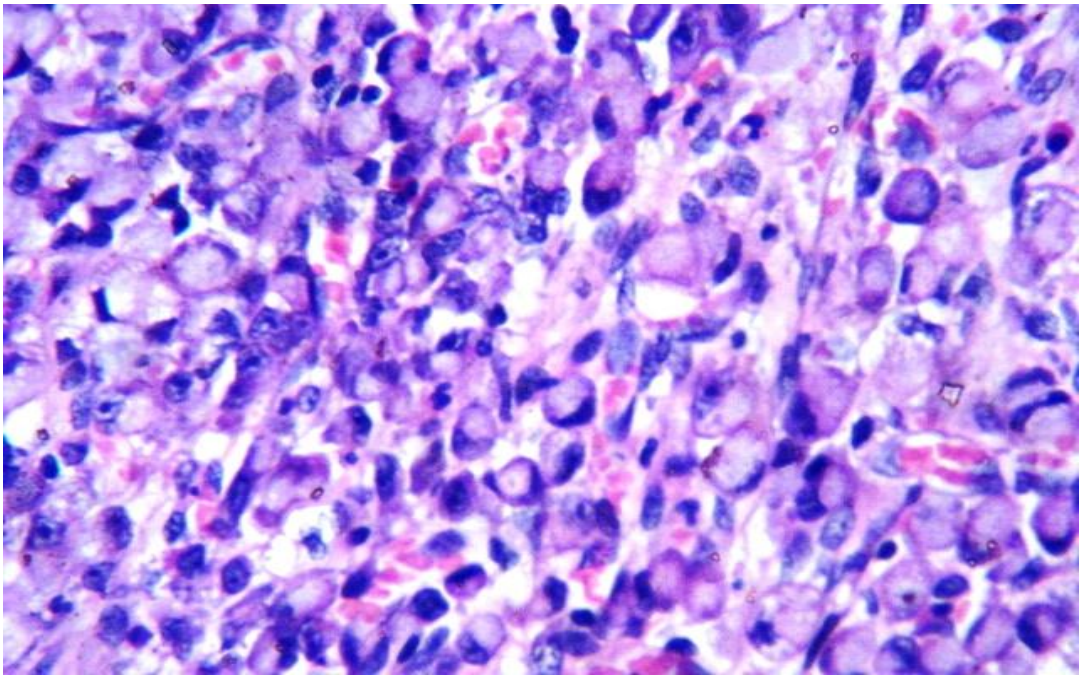


FIG 16 ; Signet ring cell carcinoma showing diffusely arranged signet ring cells with cytoplasmic mucin pushing the nuclei to periphery H&E x 400

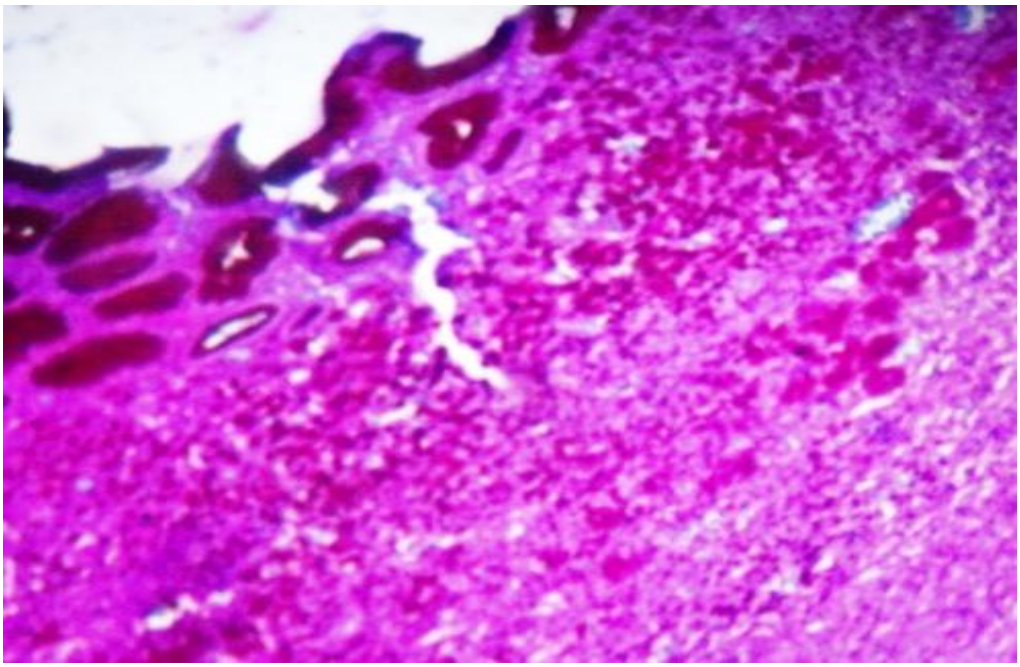


FIG 17 ; Signet ring cell carcinoma in PAS X 100

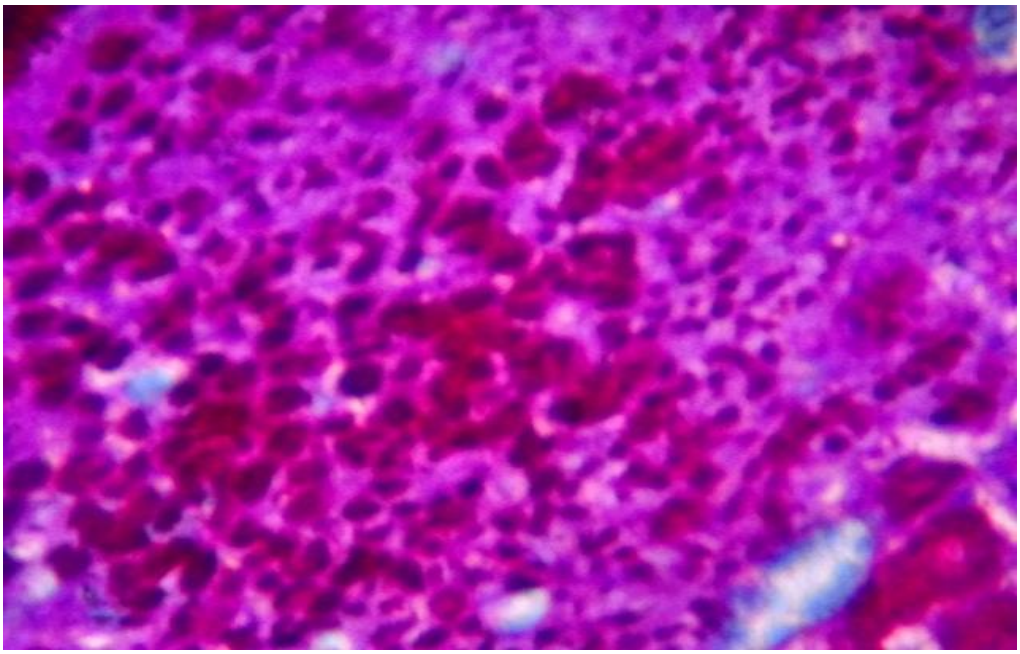


FIG 18 ; Signet ring cell carcinoma in PAS stain (Periodic acid Schiff stain) showing neutral mucin in diffusely arranged signet ring cells X 400

Table 15; Immunohistochemical analysis in gastric carcinoma

S.NO	HPE .NO	AGE/SEX	REPORT	IHC DONE	EXPRESSION
1.	2030/09	60/M	Mucinous adenocarcinoma	MUC 2	+++
2.	2783/09	48/M	Poorly differentiated adenocarcinoma	MUC 2	Negative
3.	1377/10	51/F	Mucinous adenocarcinoma	MUC 2	+++
4.	2834/10	60/M	Moderately differentiated adenocarcinoma	MUC 2	Intestinal metaplasia +
5.	2951/10	60/F	Squamous cell carcinoma	AE1/AE3	+
6.	3583/10	47/M	Signet ring cell carcinoma	MUC 2	++
7.	4059/10	50/F	Poorly differentiated adenocarcinoma	MUC 2	Negative
8.	1162/11	65/M	Moderately differentiated adenocarcinoma	MUC 2	Intestinal metaplasia +
9.	1287/11	64/M	Well differentiated adenocarcinoma	MUC 2	Negative
10.	1826/11	50/M	Well differentiated adenocarcinoma	MUC 2	Intestinal metaplasia +

For MUC2 , the control was small intestinal goblet cells fig (23,24)

MUC 2 stained the perinuclear zone in goblet cells of intestinal metaplasia, fig (25,26) diffuse cytoplasmic staining in malignant cells and also stained >50% of extracellular mucin in mucinous adenocarcinoma. fig(31,32,33,34)

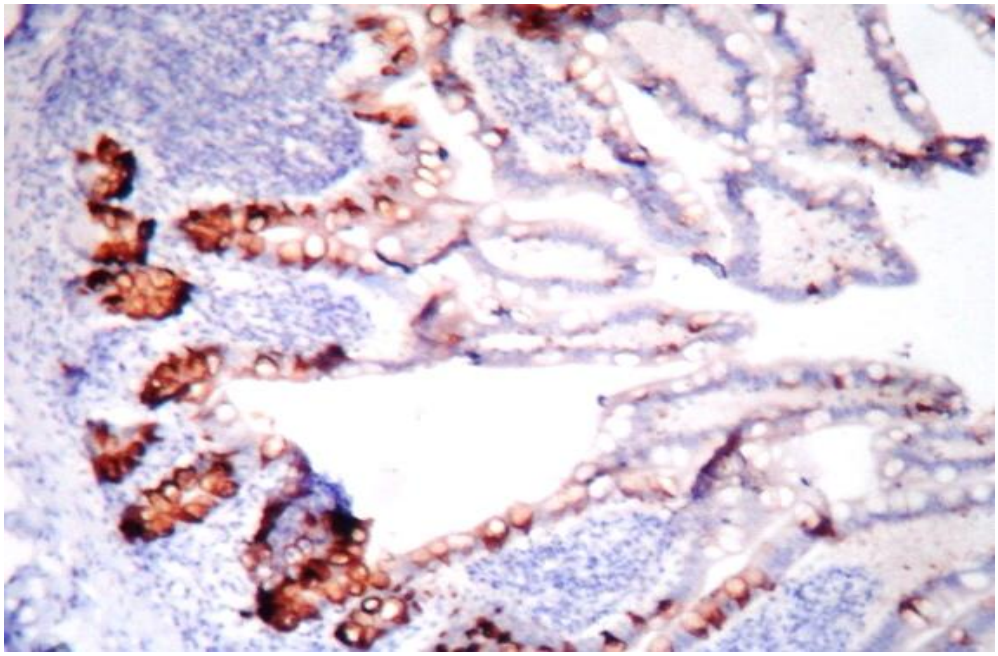


FIG 23; Intestinal goblet cells expressing MUC 2 positivity – control X100

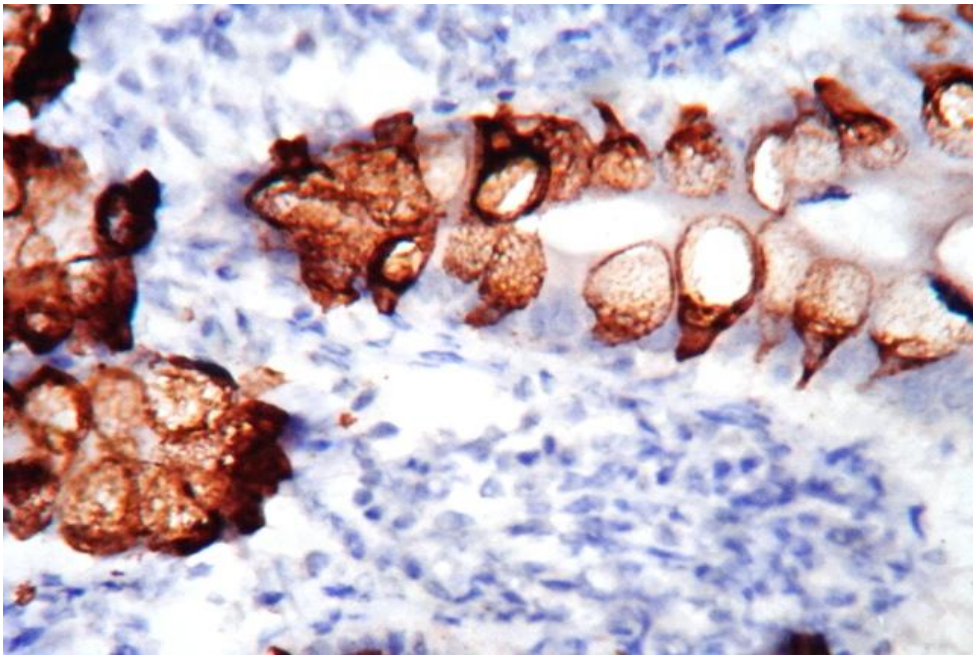


FIG 24 ; Goblet cells in intestinal epithelium expressing MUC 2 in perinuclear zone x400

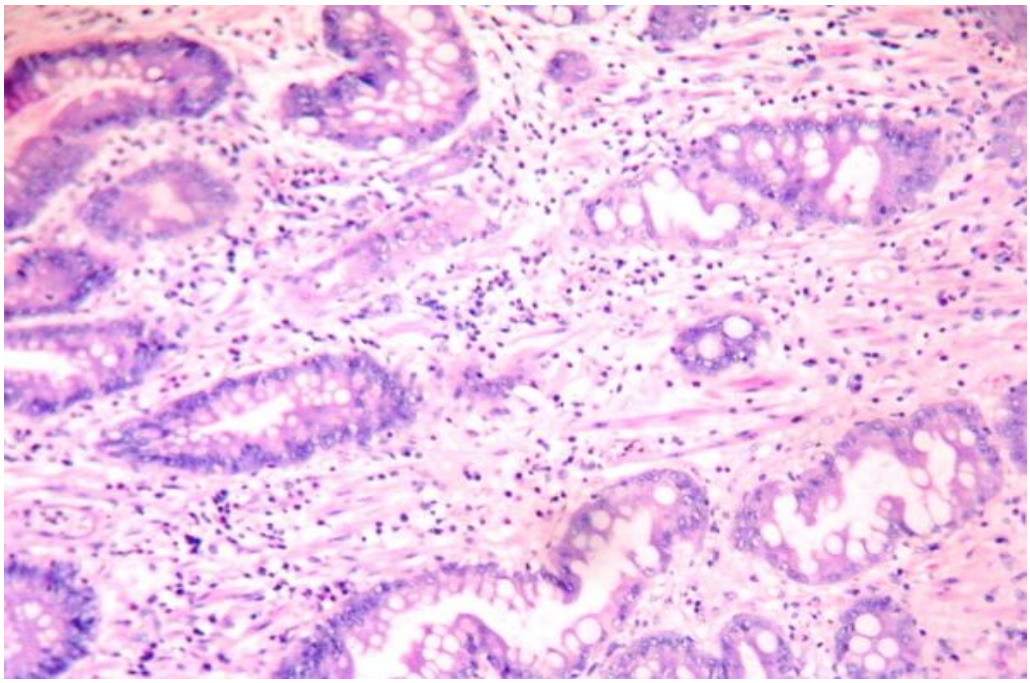


FIG 25 ; Gastric mucosa with intestinal metaplasia in H&E X 100

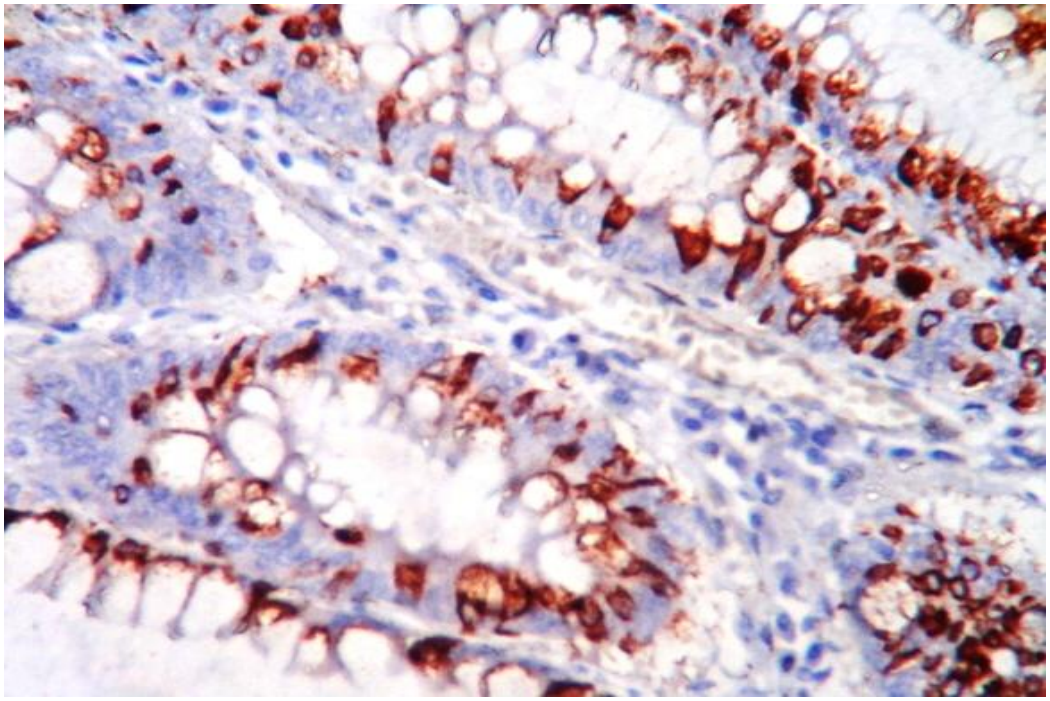


FIG 26 ; Intestinal metaplasia showing MUC 2 positivity in perinuclear zone of goblet cells x400

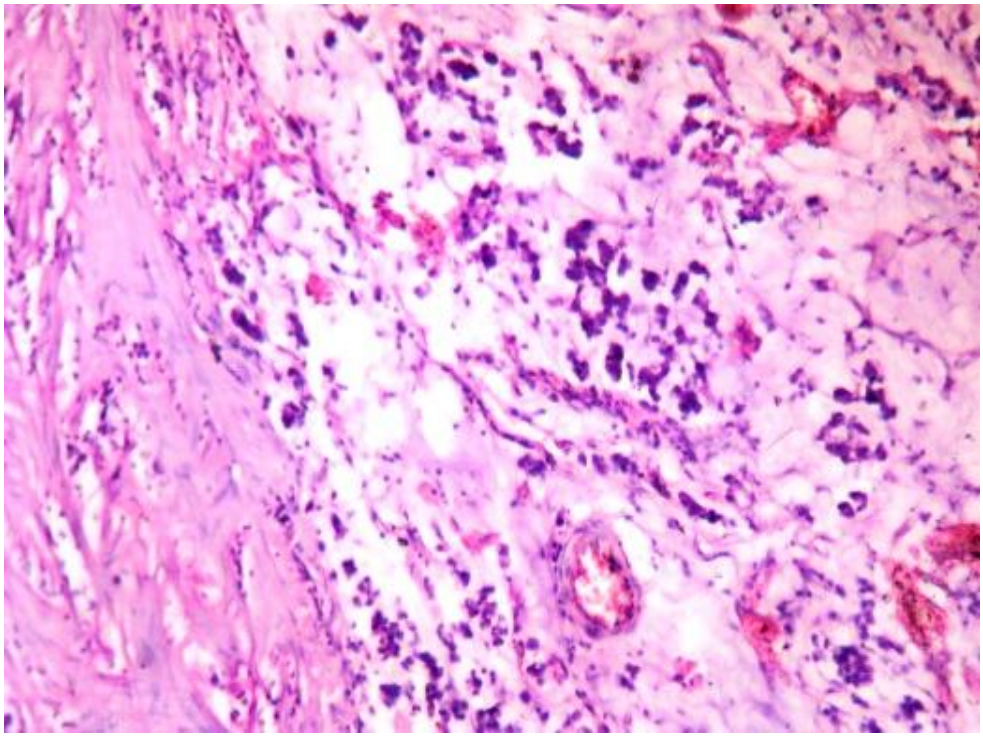


FIG 31 ; Mucinous adenocarcinoma showing malignant cells in mucinous pool H&E X100

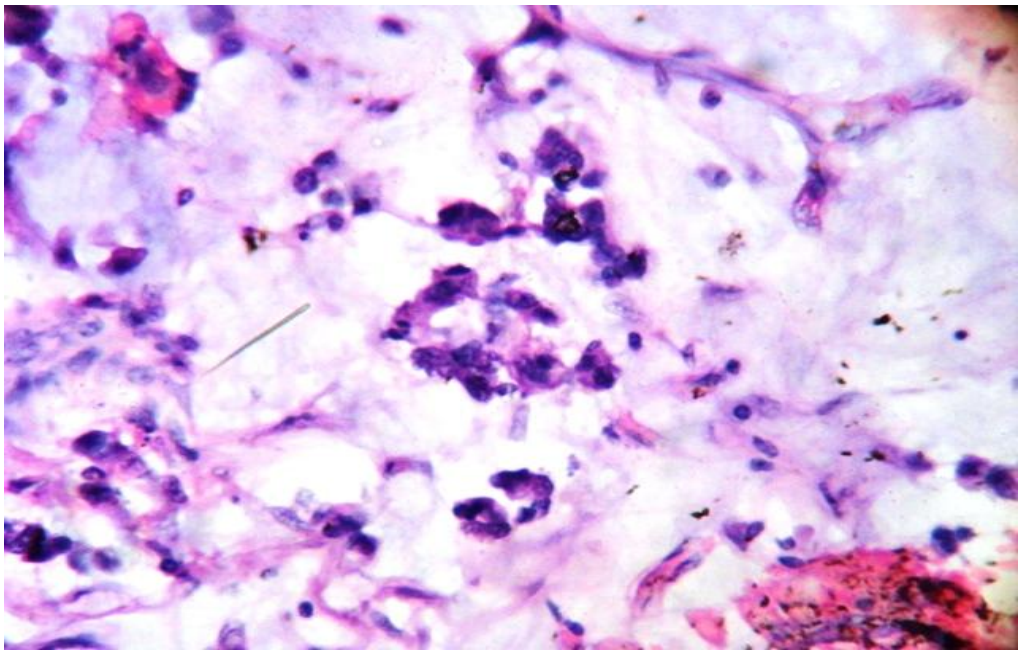


FIG 32 ; Mucinous adenocarcinoma showing malignant cells in mucinous pool H&E X400

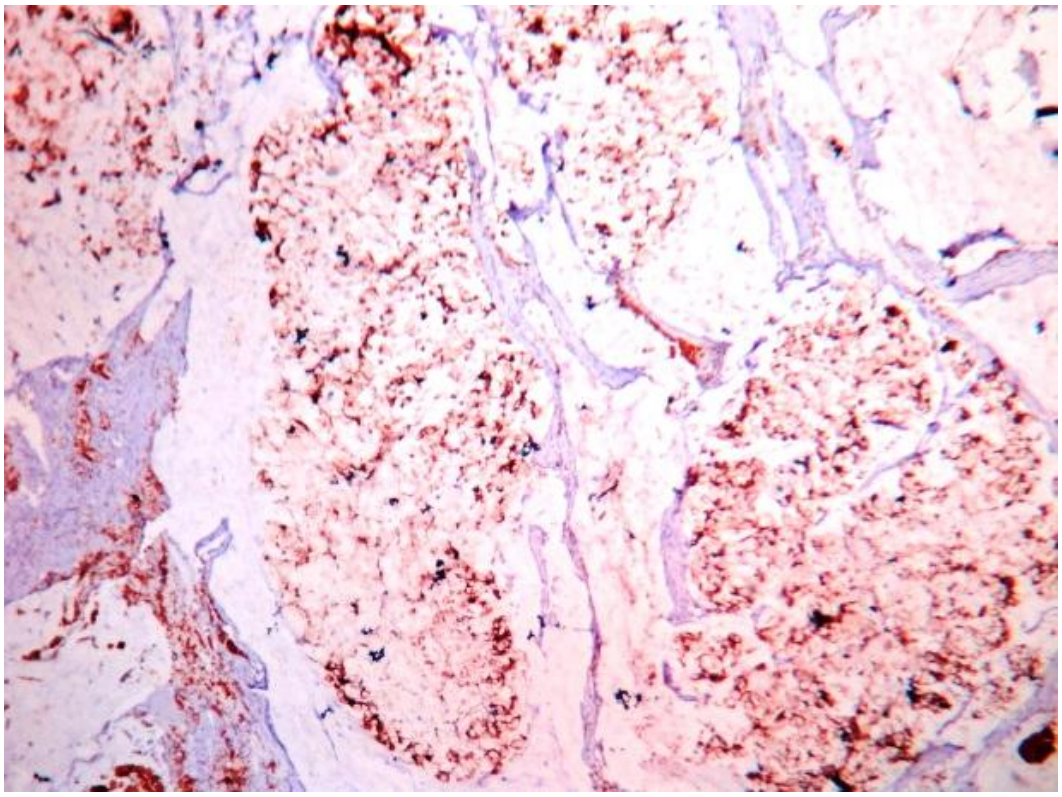


FIG 33 ; Mucinous adenocarcinoma showing MUC 2 Positivity in extracellular mucinous pool X100

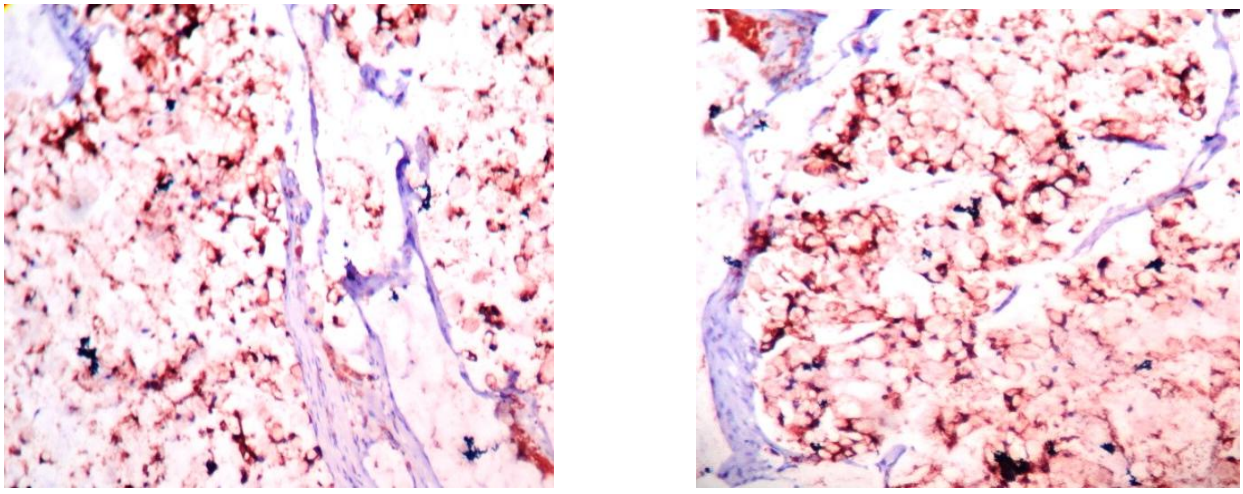


FIG 34(a,b) ; Mucinous adenocarcinoma showing MUC 2 Positivity in extracellular mucinous pool X400

Subramani Duraibabu et al,[73] studied expression of MUC 2 by semiquantitative approach.

In it 100 cells in 5 different fields should be counted and the mean should be taken.

Results :

Negative	(-)
Few positive (< 25%)	(+)
Well defined area with positive cells (25% -50%)	(++)
Extensive area with positive cells (59%-75%)	(+++)
Most cells are stained (>75%)	(++++)

k.kawaguchi et al , studied in signet ring cell carcinoma staining of >10% of cancer cells was classified as positive expression. <10% were classified as negative expression for MUC 2. Fig (35,36)

Samuel et al[67], MUC2 is commonly expressed in intestinal type of gastric adenocarcinoma. Wang rongquan et al[79],studied MUC2 expression is seen in well and moderately differentiated adenocarcinoma. The expression is decreased in poorly differentiated and variable in signet ring cell carcinoma. In this study, poorly differentiated adenocarcinoma showed negative expression for MUC 2 fig (29,30)

According to Liu Q etal[94], Nguyen etal[55], connel et al[95], MUC 2 expression in gastric adenocarcinoma varied from 0-50% of cases. In this study, intestinal type of adenocarcinoma is negative for MUC 2 expression.fig(27,28)

AE1/AE3 showed diffuse and strong cytoplasmic positivity in Squamous cell carcinoma. Fig (19,20,21,22)

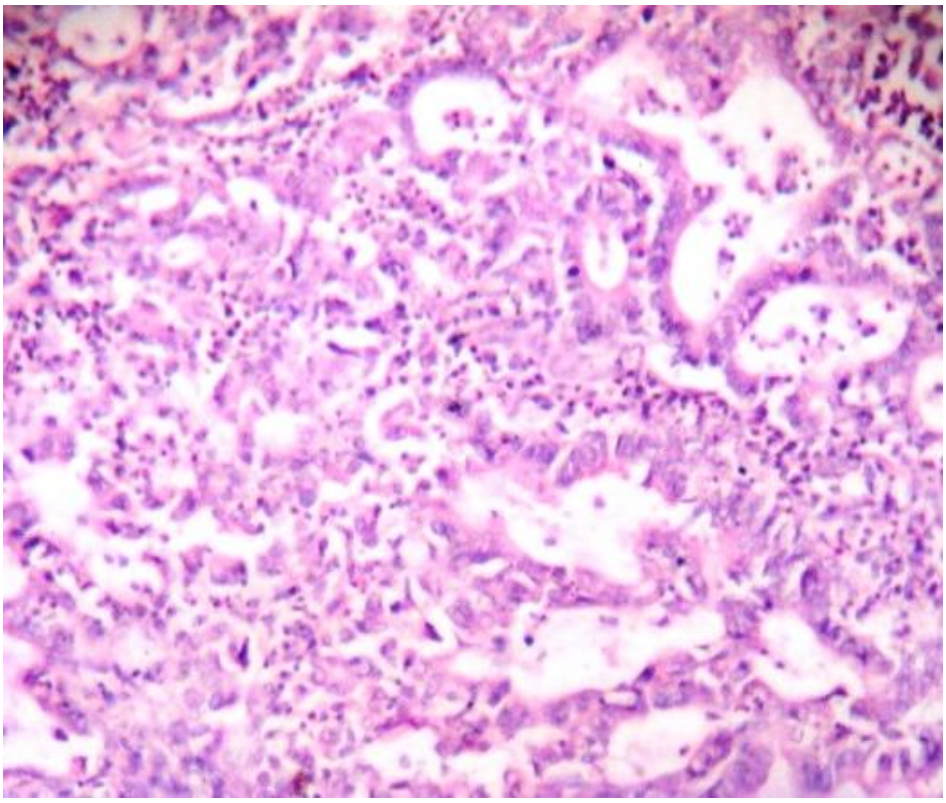


FIG 27 ; Intestinal type of adenocarcinoma showing tubular pattern in H&E X100

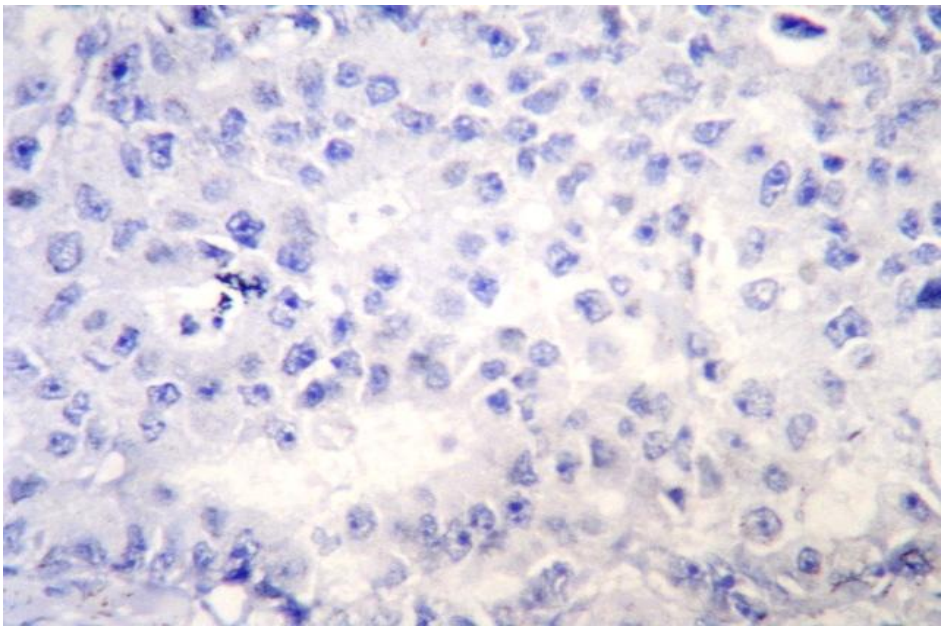


FIG 28 ; Negative MUC 2 expression in intestinal type of adenocarcinoma

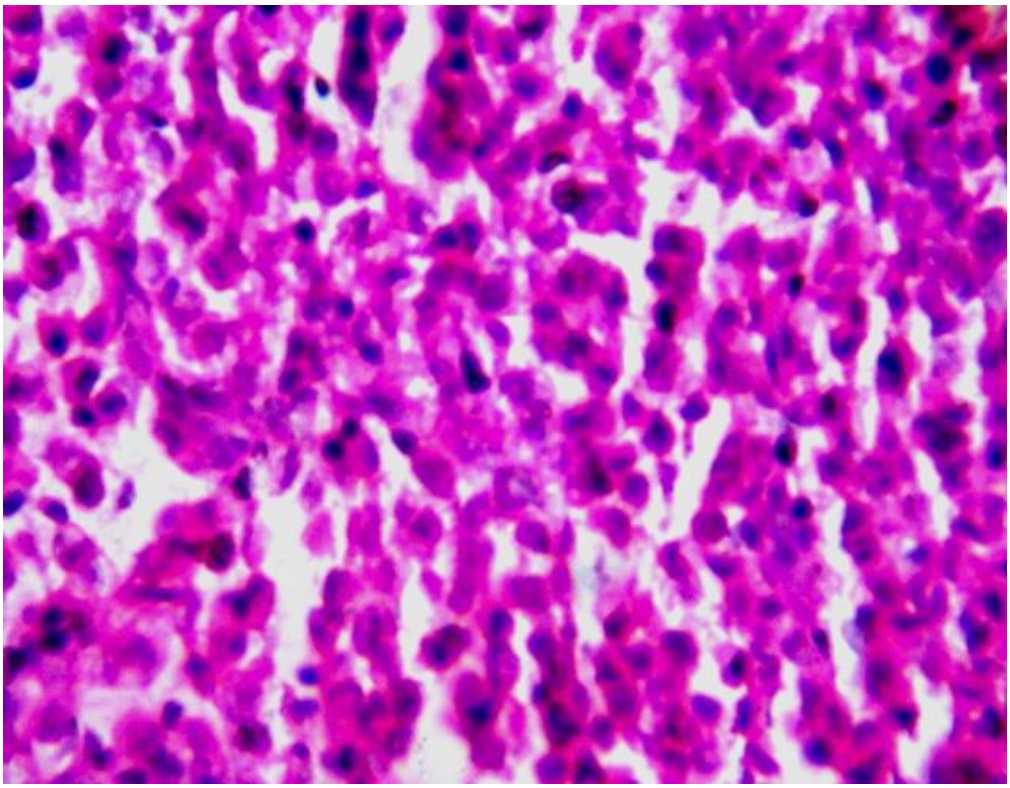


FIG 29 ; Poorly differentiated adenocarcinoma in H&E x 400

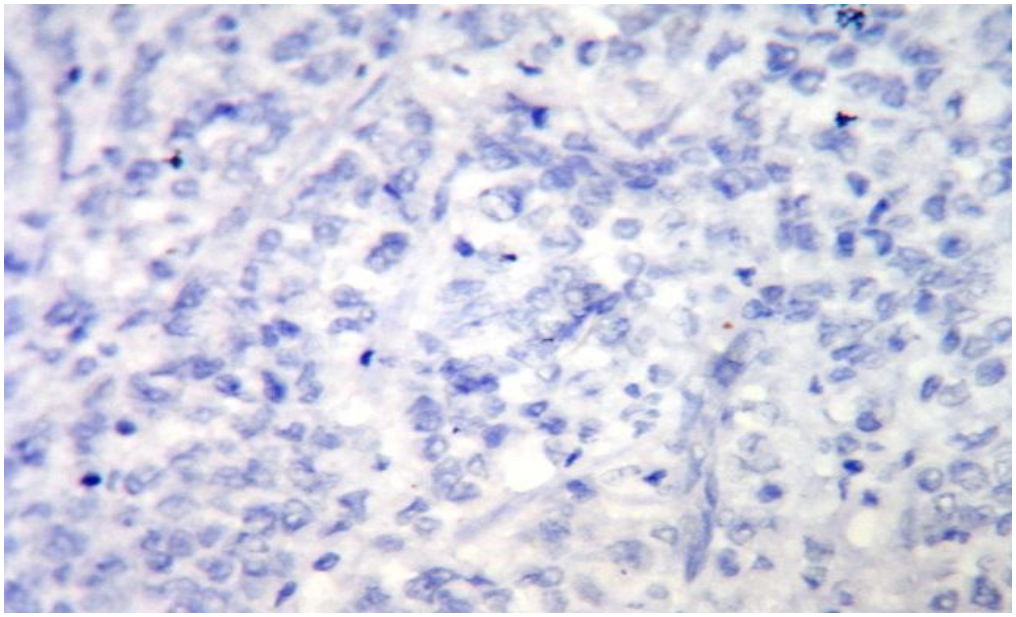


FIG 30 ; Negative MUC 2 expression in poorly differentiated adenocarcinoma

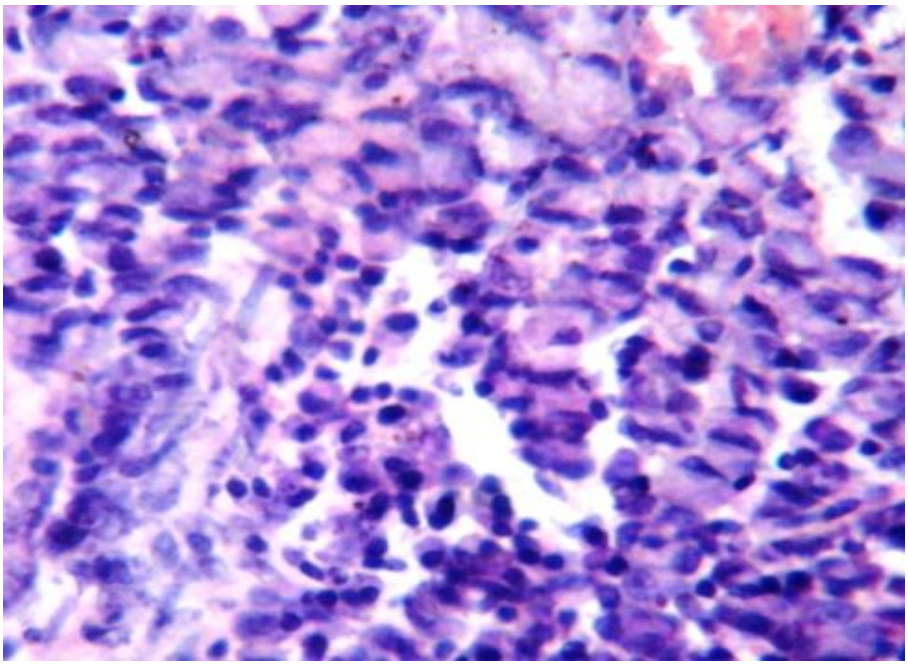


FIG 35 ; Signet ring cell carcinoma with signet ring cells H&E X 400

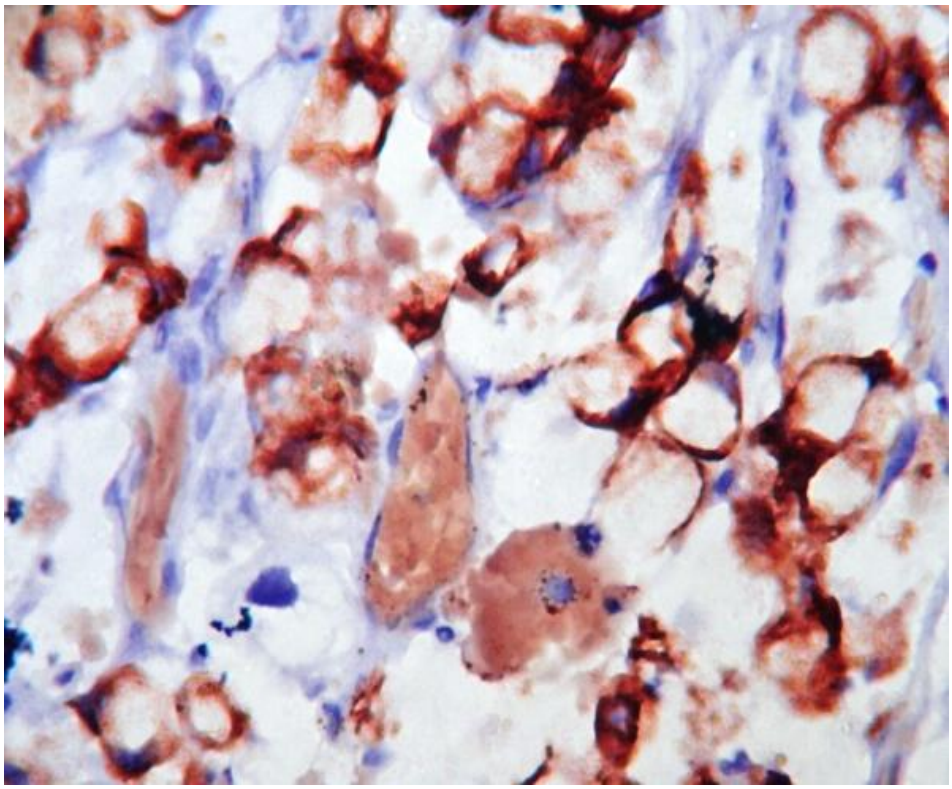


FIG 36 ; Signet ring cell carcinoma with signet ring cells expressing MUC 2 positivity X 400

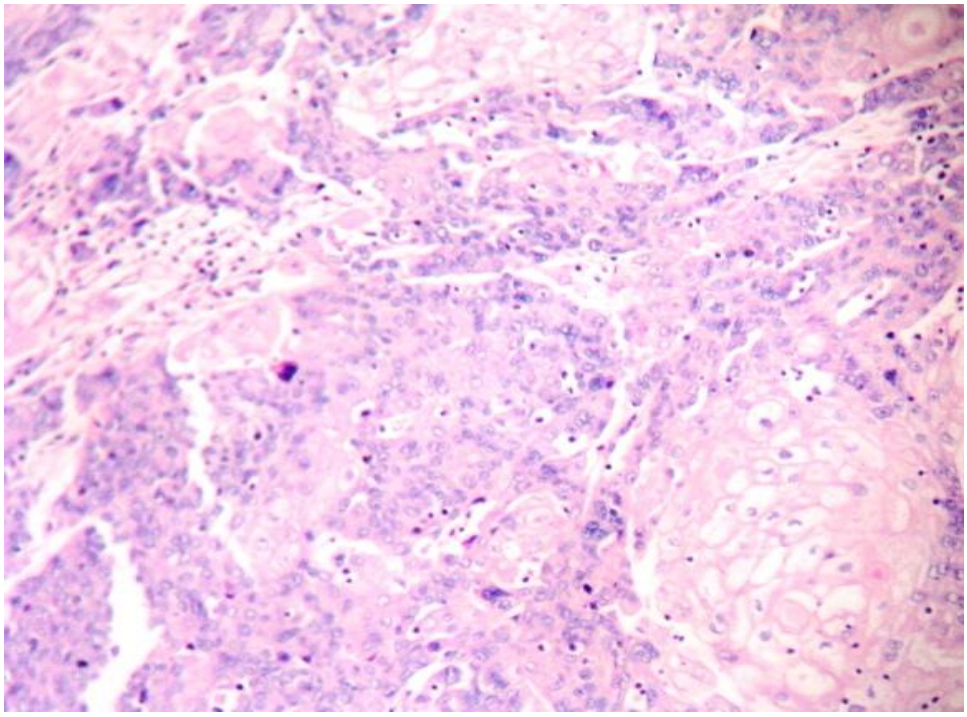


FIG 19 ; Squamous cell carcinoma with malignant keratin pearl in H&E x100

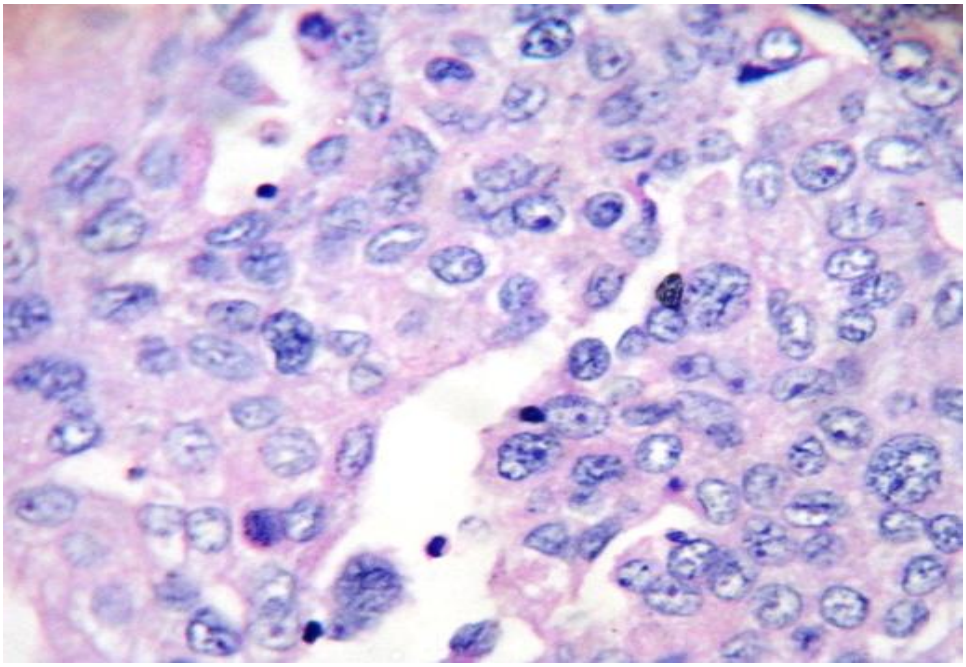


FIG 20 ; Squamous cell carcinoma showing malignant cells in H&E x 400

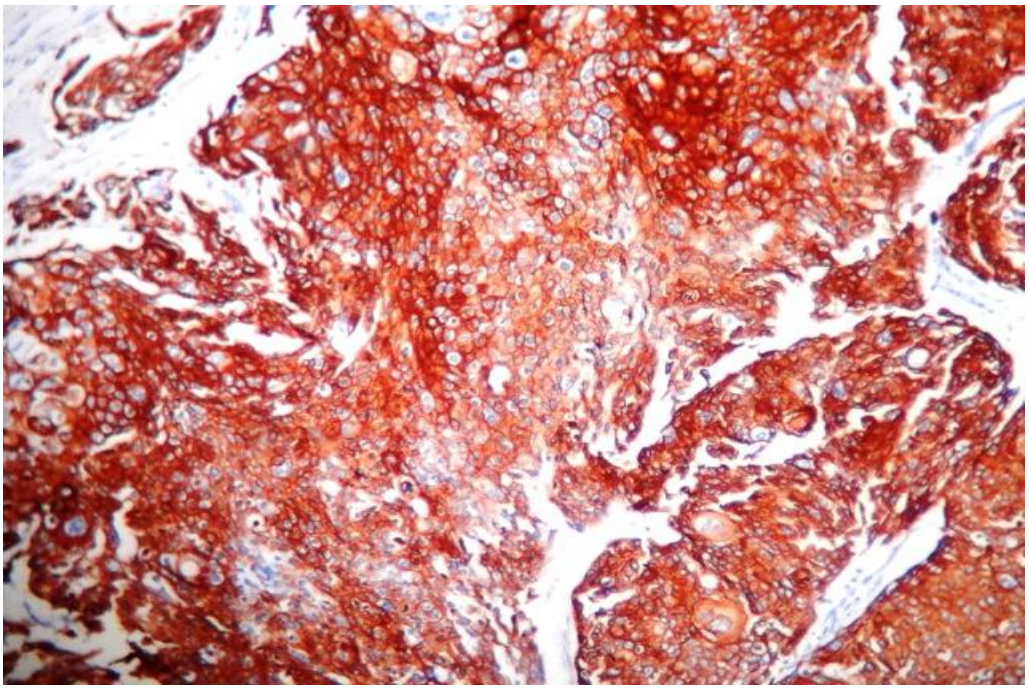


FIG 21 ; Squamous cell carcinoma expressing diffuse cytoplasmic positivity for AE1/AE3 X100

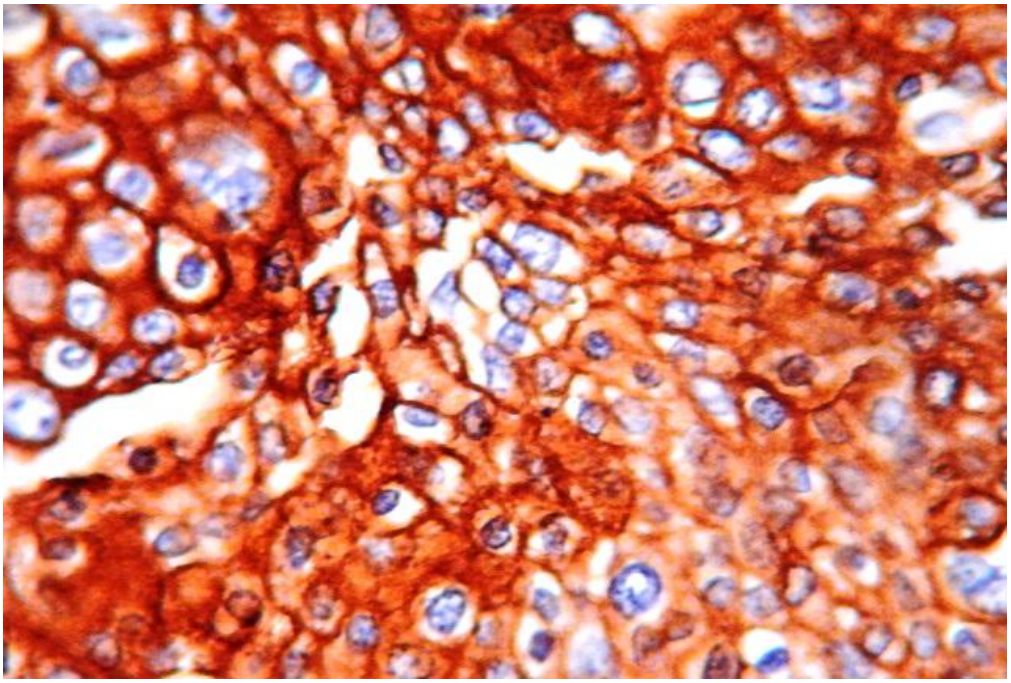


FIG 22 ; Diffuse cytoplasmic positivity of AE1/AE3 in malignant squamous cells X400

DISCUSSION

Gastric cancer is the 2nd most common cancer worldwide constituting 50% of all the gastrointestinal cancer[46]. It is more common in low socioeconomic groups and 60% occurs in developing countries. Highest incidence is in East Asia, East Europe and some part of South Africa and lowest incidence is in North America[65].

Table 16; Comparison of sex wise distribution of gastric cancer with various studies

STUDIES	MALE	FEMALE	TOTAL
Hidetsugu yamagishi et al[24]	42	21	63
Shigang ding et al[69]	41	10	51
Lei hung et al[42]	38	11	49
Jiangdong wang et al[60]	47	15	62
Kataya gudis et al[39]	86	43	129
Xiao ping et al[83]	40	17	57
Young euncho et al[87]	91	47	138
j. maria D begnami et al[45]	64	36	100
Ok jae lee et al[61]	72	34	106
Jiro nakamoto et al[31]	78	30	108
IN THIS STUDY	29	21	50

This comparison showed that there was male predominance in gastric cancer. From above data , the common M:F was 4:1, in this study M:F was 3:2.

Table 17; comparison of mean age group,

STUDIES	MEAN AGE WITH AGE RANGE
Do youn park et al[17]	61yrs
Leihung et al[42]	61.6 ± 8 yrs
Young guncho et al[87]	59 yrs [23-84]
Zhong zheng zhao et al[93]	61 yrs [30-91]
Ok jae lee et al[61]	57.8 yrs
In this study	56.7 yrs[25 - 80]

Most of them had their mean age as 61,59,57 yrs. In this study, the mean age was 56.7 yrs for both male and female patients

In Thanjavur Medical College , during the period October 2008 - September 2011 a total of 13,593 specimens were received. It include 303 gastric biopsies and 50 gastrectomy specimens.

Among total specimens received, 316 cases were reported as cancers of gastrointestinal tract. Of which ,195 cases were reported as gastric carcinomas Thus percentage of gastric carcinoma cases among gastrointestinal cancers was 61.70%

Table 18;

Comparison of incidence of gastric cancer between Thanjavur Medical College and Sri devaraj urs Medical college, kolar, Karnataka.

Comparing datas	**Sri devaraj urs medical College,kolar,Karnataka [Jan 1997 – Dec 2006][63]**	**Thanjavur medical college [Oct 2008 - sep2011]**
Total no of cases received	19,615	13,593
Total malignancy	2744 (13.98%)	4424 (32.5%)
Gastrointestinal Cancer	630 (22.96%)	316 (7.14%)
Gastric cancer	305 (48.4%)	195 (61 .70%)
Male : female	1:0.5	3:2
Commonest decade	6^{th} and 7^{th} decade	6^{th} decade

R.kalyani et al[63] studied that , In sri devaraj urs medical college,kolar,karnataka, a total of 19,615 cases were received for histopathological examination during the period

Jan 1997 –Dec 2006. Of them 2744 cases[13.98%] were malignancy. Of the malignancy 630 cases [22.96%] were gastrointestinal tract malignancy. Among GI malignancy , the most common site is stomach. Of 630 cases, 305 cases were stomach cancer[48.4%].

In this study , 13,593 cases were received during the period oct 2008-sep2011. Of them 4424(32.5%) were reported as malignancy. Of the malignancy, 316(7.14%) cases were gastrointestinal malignancy. Of which 195 (61.70%) were gastric cancer.

Table 19;

Comparison of age wise and sex wise distribution of gastric carcinoma in gastrectomy specimens with sri Devaraj urs medical college ,kolar, karnataka.

AGE	SRI DEVARAJ URS MEDICAL COLLEGE [JAN 1997- DEC2006][63]		THANJAVUR MEDICAL COLLEGE [OCT 2008 – SEP2011]	
	MALE	FEMALE	MALE	FEMALE
20-29	7	7	0	2
30-39	10	7	2	1
40-49	37	18	5	5
50-59	51	29	11	8
60-69	66	27	9	5
70-79	24	8	1	0
80-89	7	7	1	0
TOTAL	202	103	29	21

This shows that gastric cancer was predominantly present among male patients

with M:F ratio in this study was 3:2, in sri devaraj urs medical college it was 1:0.5 The peak incidence of gastric cancer in this study was in 6th decade where as in Sri devaraj urs medical college it was in 7th and 6th decade and 40% seen between 50-70 years.

Table 20;

Comparison of incidence[63] of gastric cancer among total cancers with other areas.

AREAS	INCIDENCE
BANGALORE	13.6%
BHOPAL	5.8%
CHENNAI	14.9%
DELHI	3.9%
MUMBAI	6%
IN OUR STUDY	4.4%

From the registry, the incidence of gastric cancer among the overall malignancy in Various cities during the period 1987 - 2003 were shown and compared with the Incidence of the same in our institution from 2008 OCT -2011 SEP.

Kamala krishnaswamy et al[36] studied that in India gastric cancer was more common in the southern states as well as in Kashmir. Though H.pylori infection was an important risk factor, salted food and poor dietary habits can also inflict damage. In Tamilnadu, the incidence of gastric cancer was high due to high consumption of salt.

In Kashmir, intake of salted tea and habit of consuming sun dried foods which promote nitrosocompound formation. Intake of vegetables – Brassica ,spices were

rich source of nitroso compounds. R. Kalyani et al[63], studied that in India, gastric cancer was more common in south India which include Hyderabad, Nellore, Thiruvallur, Erode, Kasaragod, Palakkod, Kancheepuram and south karnataka.

Table 21;

Comparison with types by Lauren's classification ,

Studies	Intestinal type	Diffuse type	Total cases
Kabashima et al[4]	40	20	60
Shigang ding et al[69]	35	16	51
Mikhail lisovsky et al[50]	33	44	77
Jaing dongwang et al[60]	40	22	62
Lei hung et al[42]	32	17	49
In this study	30	19	49

In this study, of 50 cases, 49 cases were reported as adenocarinoma and one case was reported as squamous cell carcinoma. Of which intestinal type adenocarcinoma constitutes 61.22% and the diffuse type constitutes 38.78%. This shows that intestinal type was more common compared to diffuse type.

Table22;

comparison of cases with average age group in Lauren's classification

Studies	Average age - Diffuse Type	Average age – Intestinal type
Kabashima et al[4]	54.4 ± 9.1	55.5 ± 11.5
In this study	53.2	54 .3

Thus the average age for both types of gastric cancer was around 54 -55 years

Table 23;

Comparison of localisation of gastric tumor

Studies	Cardia	Middle	Antrum	Total cases
Kataya gudis MS et al[39]	41	39	49	129
Charles M et al[12]	58	0	46	104
Kabashima et al[4]	3	34	28	60
In this study	6	1	43	50

From the above comparison, most of the gastric cancer arised from antrum of the stomach, followed by cardia and fundus region. In this study, 86% from antrum,12% from cardiac region and 2% from middle region of stomach.

Nubia munoz et al[58], showed that incidence of gastric cancer at gastric cardia was increased now a days and It was more prevelant in canada, USA. Still the most common site was antrum in our study.

Table 24; Comparison of gastric tumors by differentiation

Studies	Well differentiated adenocarcinoma	Moderately Differentiated adenocarcinoma	Poorly differentiated adenocarcinoma	Total Cases
Emmanuelle Leteurtre et al[18]	2	11	18	31
Shigang ding et al[69]	19	22	10	51
In this study	7	22	17	46

From the above comparison, most of the tumors were moderately differentiated, comprising around 44.8% of the differentiated carcinomas. Well differentiated constitutes around 14.2%, poorly differentiated constitutes around 36.4% in this study.

Table 25;

Comparison according to Japanese society classification,

studies	papillary	tubular	Poor	Signet ring cell	mucinous	Total
Hua chuan Zheng et al[26]	2	208	117	43	2	372
Zhong sheng Zhae et al[93]	16	226	100	65	29	436
Min .a.kim Et al[51]	0	425	439	139	55	1058
Min sung kim Et al[52]	0	94	105	38	11	248
In this study	0	29	17	1	2	50
In this study , one case was reported as squamous cell Carcinoma					1	

Thus from the above comparison most of the cancers were tubular type around 58% in this study followed by poorly differentiated cancer was 34% and the signet ring cell type was 2%.

Table 26;Comparison according to early and advanced cancer

Studies	Early cancer	Advanced cancer	total
Do youn park et Al[17]	86	56	142
Yoo ri kim et Al[86]	2	27	29
In this study	1	48	50

Thus 98 % 0f the cancers were advanced cancers

Table 27;

Comparison among differentiated and undifferentiated carcinomas

Studies	Differentiated	undifferentiated	total
Hiroaki takahashi et al[25]	89	13	102
Jiro nakamoto et al[31]	79	29	108
In this study	31	19	50

From above comparison most of them were differentiated (62%) and the undifferentiated cancers were (38%)

Table 28;

Comparison according to WHO classification.

studies	papillary	tubular	mucinous	Signet ring cell	others	Total cases
Emmanuelle Leteurtre et al[18]	0	14	3	12	2	31
In this study	0	46	2	1	1	50

This shows that 92% of the cancer were from tubular type , 4% mucinous, 2% signet ring cell type and 2% was by others.

Nubia munoz et al[58], studied in 1990, stomach cancer was the second most common cancer in world after lung cancer. About 800,000 (10%) cases were diagnosed. of which 60% were in developing countries. Steady decline in rates have been observed everywhere in the last few decades but the absolute number of new cases per year is increasing because of aging of the population. The overall mortality rate is around 70%-90% where as in Japan it is around 40%. India has lowest risk of gastric cancer (<15/100,000)

SPECIAL STAIN STUDY

Gastrectomy cases were evaluated for mucin histochemistry by using combined Alcian Blue pH2.5 PAS and PAS [Periodic acid Schiff stain].

Acid mucin was expressed in 48 cases [96%], signet ring cell carcinoma was stained by PAS shows neutral mucin expression.

IMMUNOHISTOCHEMISTRY

MUC 2 IN INTESTINAL METAPLASIA

Samuel b et al[67] ,studied that in intestinal metaplasia, MUC2 is expressed in supranuclear region of goblet cells due to compression of cytoplasm by mature mucous granules in goblet cells.

In this study, MUC 2 was expressed in supranuclear region of goblet cells in intestinal metaplasia.

MUC 2 IN DIFFERENTIATED GASTRIC ADENOCARCINOMA

Samuel et al[67],MUC2 is commonly expressed in intestinal type of gastric adenocarcinoma .Wang rongquan et al[79],studied MUC2 expression is seen in well and moderately differentiated adenocarcinoma. The expression is decreased in poorly differentiated and variable in signet ring cell carcinoma.

According to Liu Q etal[94], Nguyen etal[55], connel et al[95], MUC 2 expression in gastric adenocarcinoma varied from 0-50% of cases. In this study, intestinal type of adenocarcinomas were negative for MUC 2 expression.

MUC 2 IN MUCINOUS ADENOCARCINOMA

Celso . A et al[11] , studied that MUC 2 expression is more in mucinous adenocarcinoma of stomach. According to WHO, if there is > 50% of MUC2 expression then it is called mucinous adenocarcinoma.MUC 2 is expressed in both intracellular and extracellular mucin.

In this study, MUC 2 was expressed in >50% of mucinous area in mucinous adenocarcinoma.

MUC 2 IN SIGNET RING CELL CARCINOMA OF STOMACH

Meng meng tian et al[46],studied MUC2 expression has significantly higher lymph node metastasis rate and vascular invasion than MUC 2 negative signet ring cell carcinoma cases.MUC 2 expression also increased in those signet ring cell tumor having deeper wall invasion and higher TNM stage. No significant correlation was found between MUC 2 expression, age and distant metastasis. Gastric signet ring cell carcinoma expressing intestinal phenotype markers (GI , I TYPE) has significantly lower survival rates than those without expression.

In this study , signet ring cell carcinoma showed positivity for MUC 2.

MUC 2 - ROLE IN PROGNOSIS OF GASTRIC ADENOCARCINOMA

Yusuki tajima et al[89], studied that MUC2 expression indicates mucosal carcinoma and inversely associated with submucosal invasion.

Kabhashima et al[4] , studied that G – phenotype of gastric carcinoma can potentially degrade the extracellular matrix through the overexpression of matrix metalloproteinase compared with intestinal type of gastric adenocarcinoma. Thus gastric phenotype has poor prognosis than that of intestinal type.

Shibata et al[91],reported that G phenotype has lower apoptotic index / proliferative index ratio

than that of I –phenotype of gastric cancer. He studied that gastric adenoma associated with MUC 2 expression than with advanced cancer. I phenotype was highly associated with gastric adenoma than early and advanced gastric cancer.

k.kawaguchi et al[35], studied gastric phenotype cancer are considered to have greater invasiveness and metastastic potential than intestinal phenotype of gastric cancer.

Minh d.nguyen et al[55], studied MUC 2 expression is variable in signet ring cell carcinoma of Stomach.

Jiro nakamoto et al[31], G phenotypic expression in submucosal carcinoma have an important risk for lymph node metastasis . For I phenotype , it is measured by proliferative activity.

Ok jae lee et al[61], studied mucin phenotype may be correlated with histologic differentiation and Lauren's classification of tumor. It was quite different from those histological classification in many cases. Histologic type and Laurens classification did not have prognostic significance on multivariate analysis.I phenotypic expression of tumor was an independent good prognostic factor with lower tumor stage.

Therefore mucin phenotype may have an important role as a prognostic factor of gastric adenocarcinoma compared to conventional histological types. I phenotype has better outcome than non I type.

SQUAMOUS CELL CARCINOMA[13]

Generally squamous cell carcinoma stain diffusely and strongly with CAM 5.2, AE1/AE3, 34bE12, CK5/6, CK14 and CK19

In this study ,squamous cell carcinoma was stained with AE1/AE3 which show strong and diffuse cytoplasmic positivity.

David callacendo Riva et al[15] , studied that primary gastric squamous cell carcinoma is an exceedingly rare disease which accounts for < 0.5% of all primary neoplasm of the stomach. since 1985 there have been fewer than 100 cases published in the world literature. Gastric squamous cell carcinoma occurs mostly in male with M:F ratio of 5:1 and peak incidence at 6th decade of life

According to David Callacendo Riva et al[15], to differentiate pure gastric squamous cell carcinoma from extension or metastasis, 3 diagnostic criteria must be met,

1. The tumor must not be located in the cardia
2. The tumor must not extend into esophagus
3. There should be no evidence of squamous cell carcinoma in any other part of the body

The pathogenesis for squamous cell carcinoma of the stomach is given by 4 main theories

1. Nests of ectopic squamous cells in gastric mucosa
2. Squamous metaplasia of gastric mucosa before malignant transformation
3. Squamous differentiation in a preexisting adenocarcinoma
4. Multipotential stem cells in the gastric mucosa

Squamous metaplasia occurs in healing gastric ulcer and a variety of conditions with long standing chronic inflammation such as corrosive gastric acid burns, chronic inflammation in Menetrier disease, after chemotherapy for well differentiated lymphocytic lymphoma.

SUMMARY AND CONCLUSION

- From the period 2008 oct – 2011 sep, 50 cases of gastrectomy specimens were analysed Age, sex and site of the lesion were recorded. Subtyping of carcinoma was done. Mucin type neutral / acidic is identified by AB pH 2.5 PAS and PAS staining
- Immunohistochemistry using MUC2 primary antibody was done to assess the role of its expression in various types of gastric adenocarcinoma. Results were tabulated and analysed.

From endoscopic biopsies

- Incidence of gastric cancer among gastric endoscopic biopsies - 47.8%
- Gastric cancer in male among gastric endoscopic biopsies – 48.2%
- Gastric cancer in female among gastric endoscopic biopsies - 46.6%
- Male predominate in the ratio of 3:1
- Male peak incidence in the 6^{th} decade
- Female peak incidence in the 5^{th} decade

From gastrectomy specimen

- Incidence of gastric cancer among the malignancies during the period 2008 oct – 2011 sep is 4.4%
- Gastric cancer in male among gastrectomy cases– 58%
- Gastric cancer in female among gastrectomy cases - 42%
- Male predominate in the ratio of 3:2
- Male peak incidence in the 6^{th} decade
- Female peak incidence in the 5^{th} decade
- Mean age of gastric cancer – 56.7yrs(25-80)

- Incidence of early gastric cancer - 2%
- Commonest site - antropyloric region 86%
- Intestinal type predominates by 61.2% with male predominance
- Tubular carcinoma occur frequently about 92% in both sexes
- Incidence of signet ring cell carcinoma – 2%
- On mucin histochemistry, acid mucin is demonstrated in - 96 % of gastric cancer.
- Acid mucin is expressed more in poorly differentiated and mucinous adenocarcinoma type of gastric cancer
- MUC 2 expression is more in intestinal metaplasia, >50% in mucinous adenocarcinoma, >10% in signet ring cell carcinoma, absent in intestinal type of gastric adenocarcinoma and poorly differentiated adenocarcinoma
- AE1/AE3 showed diffuse and strong positivity in squamous cell carcinoma.

Though endoscopic facilities and immunohistochemical studies were available, the detection rate for early gastric cancer was only 2%. This emphasizes the need for active screening programs for early detection , management and preventing the progression to advanced stage of gastric cancer.

APPENDIX I

HEMATOXYLIN AND EOSIN STAIN

Preparation of the solution :

Harris hematoxylin:

Distilled water - 1000ml

Ammonium alum - 100g

Haematoxylin - 5g

Absolute ethyl alcohol - 50ml

Mercuric Oxide - 2.5g

100g of ammonium alum is dissolved in 1000ml of distilled water by heating and shaking at 60°C. Add solution of 5g of haematoxylin in 50ml of ethyl alcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 2.5g of Mercuric oxide. Mix by swirling gently.

EOSIN STAIN

Eosin Y - 1g

Distilled water - 20ml

95% ethanol - 80ml

Glacial acetic acid - 0.2ml

Dissolve 1g eosin Y in 20ml of water add 95% ethanol and glacial acetic acid.

PROCEDURE

- Sections to water.
- Harris's hematoxylin for 15 minutes. Rinse in tap water.
- Differentiate in 1% acid alcohol – 3 to 10 quick dips.
- Wash in tap water very briefly.
- Dip in ammonia water (for 10-20 seconds) saturated lithium carbonate until sections are bright blue.
- Wash in running tap water for 10-20 minutes.
- Stain with eosin for 15 seconds to 2 minutes depending on the age of the eosin and the depth of counter stain required.
- 95% alcohol – 2 changes Absolute alcohol – at least 2 changes.
- Xylene – 2 changes. Mount in DPX mountant.

APPENDIX II

COMBINED ALCIAN BLUE pH 2.5 PERIODIC ACID SCHIFF

Preparation of stains

ALCIAN BLUE SOLUTION

a) Alcian blue - 1gm

b) 3% acetic acid

c) Schiffs reagent

Basic fuchsin 1 gm,Sodium metabisulphite, anhydrous 1 gm

Distilled water 200 ml, N/I hydrochloric acid 20 ml

Boil the distilled water; add basic fuchsin and stir, cool to 50° C. Then filter and add hydrochloric acid, cool to 25°C and add the sodium metabisulphite.

This solution is ready for use when it becomes nearly colourless, which may take up to two days in the dark.

d) 1% aqueous periodic acid

METHOD

- Dewax sections and bring to water, flood section in 3% acetic acid for 3mins
- In alcian blue solution – 5 min
- Wash in distilled water
- 1% aqueous periodic acid - 5 min
- Rinse well in distilled water
- Schiff's reagent - 15 min
- Wash in running tap water 5 - 10 min
- Stain nuclei with Harris hematoxyilin and differentiate
- Wash in distilled water
- Rinse in absolute alcohol
- Clear in xylene and mount in DPX.
- **RESULT**; ACID MUCIN – BLUE,
  NEUTRAL MUCIN - MAGENTA

APPENDIX-III

PERIODIC ACID SCHIFF TECHNIQUE

Solution required

a) 0.5% periodic acid.

b) Mayer's haemalum

c) Sulphurous acid

Sodium metabisulphite 10% 6 ml

N/I hydrochloric acid 10% 5 ml

Distilled water 100 ml

(d). Schiffs reagent

Basic fuchsin 1 gm

Sodium metabisulphite, anhydrous 1 gm

Distilled water 200 ml

N/I hydrochloric acid 20 ml

Boil the distilled water; add basic fuchsin and Stir, Cool to 50° C.

Then filter and add hydrochloric acid, cool to 25°C and add the sodium metabisulphite.

This solution is ready for use when it becomes nearly colourless, which may take up to two days in the dark. (Alternatively activated charcoal may be added to the solution, shaken and filtered). The solution becomes recoloured it should be discarded.

Technique

1) Section to water
2) Periodic acid 0.5% 5 minutes
3) Rinse in distilled water
4) Schiff's reagent 15 minutes
5) Rinse in the three fresh changes of sulphurous acid
   2 minutes in each change 6 minutes
6) Wash in running tap each changes 5 minutes
7) Counter stain in Mayer's haemalum 30 seconds
8) Wash in running tap water 5 minutes
9) Dehydrate, clear and mount

Results Neutral mucin - Magenta, Nucleus - faint grey

APPENDIX IV

IMMUNOHISTOCHEMISTRY

Preparation of gelatin coated slides:

Chrome alum - 0.05 gm

Gelatin - 0.3 gm

Distilled water - 100 ml

First chrome alum is added to distilled water and then the distilled water is heated to 60◦C. Gelatin is added slowly to the heated distilled water. Glass slides are then dipped in this solution and dried overnight.

Preparation of Tris Buffered Saline (TBS): 0.005 M TBS

Distilled water - 10 litres

Sodium Chloride - 80 g

TRIS (Hydoxymethylamine) - 6.05 g

1 M Hcl - 44 ml

Final pH is adjusted to 7.6 with either 1 M Hcl or 0.2 M Tris solution

Preparation of CITRATE buffer solution (antigen retrieval solution):

Trisodium citrate - 2.94 gm

1N Hcl - 5 ml

Distilled Water - 1000 ml

Final pH is adjusted to 6.0 with 1N Hcl.

Antigen Retrieval:

The slides are placed in citrate buffer in the coplin jar and capped. The jar is then heated in a 750 W domestic microwave oven for 15 minutes (5 minutes in low power(40), 5 minutes in medium power(60) and 5 minutes in full power(80) pausing only to top up the fluid.

Procedure adopted for IHC

1. Dewax the sections in xylene (1/2 hour, two changes) and bring sections to distilled water.
2. Antigen retrieval using TBS by Microwave oven heating

3. Cool to room temperature in running tap water for 20 minutes.

4. Bring sections to TBS for 5 minutes.

5. Drain and wipe off excess TBS around sections

6. Incubate in endogenous peroxidase blocking reagent for 15-20 minutes

7. Gently wash the slides in TBS for 5 minutes.

8. Wipe off the excess fluid and Incubate in power block for 15-20 minutes.

9. Wipe the excess fluid and incubate in Primary Antibody for 60 minutes

10.Repeat steps 4 and 5

11. Incubate in super enhancer for 30 minutes

12.Repeat steps 4 and 5

13. Incubate in secondary antibody for 30 minutes

14.Repeat steps 4 and 5

15. Incubate in DAB (Diaminio Benzidine) substrate solution for 2-10 minutes

(To prepare DAB substrate, add 1ml of Substrate buffer,

1 drop of liquid DAB, and 1 drop of Substrate DAB).

Wash in distilled water, counter stain with Haematoxylin, clear in xylene and mount with DPX.

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